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WORKS OF J. A. MANDEL

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A Text-book of Physiological Chemistry.

By Olof Hammarsten, Professor of Medical and Physiological Chemistry in the University of Upsala. Authorized translation, from the second Swedish edition and from the author's enlarged and revised German edition, by John A. Mandel, Assistant to the Chair of Chemistry, etc., in the Bellevue Hospital Medical College and in the College of the City of New York. 8vo, cloth, $4.00.

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A TEXT-BOOK
OF
PHYSIOLOGICAL CHEMISTRY.

BY

OLOF HAMMARSTEN,
Professor of Medical and Physiological Chemistry in the University of Upsala.

Authorized Translation

FROM THE AUTHOR'S ENLARGED AND REVISED THIRD GERMAN EDITION

BY

JOHN A. MANDEL,
Professor of Inorganic Chemistry and Physics, and Adjunct Professor of Physiological Chemistry in the University and Bellevue Hospital Medical College.

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PREFACE TO THE SECOND GERMAN EDITION.

AFTER the appearance of the first Swedish edition of this text-book I was asked by several colaborers abroad to provide a German translation, which was at that time impossible for several reasons. But I found it very difficult to decline a similar proposal which I received from many colleagues after the second edition appeared.

I yielded, therefore, to their expressed wishes; but I found after a time that it was impossible to obtain a translator in this special province of science, notwithstanding the unwearied exertions of my publisher. Nothing remained for me but to undertake the translation myself; hence I ask the reader's indulgence for possible idiomatic or orthographic errors.

Specialists will at once perceive that the book before them is not a complete or detailed text-book. My intention was merely to supply students and physicians with a condensed and as far as possible objective representation of the principal results of physiologico-chemical research and also with the principal features of physiologico-chemical methods of work. It seems to me that I have followed a common, practical, even if not strictly correct usage in allowing space in this book to the more important pathologico-chemical facts, although I have given the book the title Text-book of Physiological Chemistry.

The arrangement of subject-matter, which deviates considerably from that generally followed in text-books, was caused by the manner in which physiological chemistry is studied in Sweden. Here physiologico- and pathologico-chemical laboratory practice is obligatory on all students of medicine. In the arrangement of such practical work I continually kept in view that it should not consist of isolated, purely chemical or analytico-chemical problems, but that always, as far as possible, it should go hand in hand with the study of the different chapters of chemical physiology.

The study of physiologico-chemical processes within the animal body must precede the study of its component parts, its fluids and tissues; and this latter study, according to my experience, will then only inspire true interest if the study of the physiological significance of those component parts be closely pursued in connection with that of the transformations which take place in these fluids and tissues.

In view of this arrangement of subject-matter, and in order to render my book of greater interest and utility to those who do not wish to take cognizance of its analytico-chemical part, I have distinguished the latter by different setting of the type. With the exception of urinary analysis, which practically is of particular importance and which has been treated somewhat elaborately, this part in general depicts only the main points in the methods of preparation and of analytical methods. The instructor who superintends the laboratory practice and who chooses the problems for work has ample opportunity to give the beginner the necessary advanced directions, and for the more experienced student, as well as for the specialist, the excellent works of HOPPE-SEYLER, NEUBAUER-HUPPERT, and others render more explicit directions superfluous.

OLOF HAMMARSTEN.

UPSALA, *October*, 1890.

TRANSLATOR'S PREFACE TO THE FIRST AMERICAN EDITION.

Knowing the demands of the medical student and practising physician for a more extended knowledge of physiological chemistry, and at the same time knowing the lack of literature on this subject in the English language, I have been led to make a translation of this most admirable work. The subject of physiological chemistry is being more and more advanced in this country, until it will soon become an obligatory study in our medical schools, and the enlargement of the literature on the subject will greatly help its progress.

It will be seen at a glance that the work is well suited as a laboratory book, for it contains the best methods for the preparation, detection, and quantitative estimation of most of the substances found in the organism and its excretions and secretions. At the author's request I have made no additions or changes whatsoever in the manuscript, and it may seem that some of the methods described, especially those on urinary analysis, are too lengthy and troublesome for the practising physician; however, the quick or clinical methods are well described in smaller handbooks on the subject. In the work of translation I have adhered as closely as possible to the author's enlarged German edition and also the original Swedish edition, and therefore the literary errors will perhaps be pardoned.

I must here express my appreciation to Mon. A. Bourgougnon, who has kindly gone carefully over the manuscript and read the proof-sheets.

J. A. Mandel.

New York, *October*, 1893.

PREFACE TO THE THIRD GERMAN EDITION.

THE present edition, which differs from the second in the arrangement of matter, contains three new chapters. The wonderful development of our knowledge of the chemistry of the carbohydrates in recent times has made it necessary to introduce a special chapter on this subject; and as the two chief groups of organic foods, the protein substances and the carbohydrates, are treated of in special chapters, the third group, the fats, likewise has a chapter devoted to it. It also appears appropriate to treat the rather extensive subject of the chemistry of respiration in a special chapter and not, as heretofore, in connection with the blood. Another deviation from the earlier editions is that the present edition is supplied with the references to the literature, in pursuance of the request made on many sides. This edition is also thoroughly revised and enlarged according to the advancement of the science; still it was naturally impossible to incorporate into the text the various papers appearing or accessible to me during the printing of this edition.

OLOF HAMMARSTEN.

UPSALA, *April*, 1895.

TRANSLATOR'S PREFACE TO THE SECOND AMERICAN EDITION.

As the subject of physiological chemistry has been rather generally introduced into the curriculum of our medical schools, and as the first American edition was one of the few authoritative works on this important subject, I was led to prepare a second American edition from the third, revised, German edition. At the request of the author no changes or additions have been made with the exception of the incorporation of the author's Addenda into the text.

J. A. MANDEL.

NEW YORK, *October*, 1898.

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PHYSIOLOGICAL CHEMISTRY.

CHAPTER I.

INTRODUCTION.

IT follows from the law of the conservation of force and matter that living beings, plants and animals, can neither produce new matter nor new force. They are only called upon to appropriate and assimilate already existing material and to transform it into new forms of force.

Out of a few relatively simple combinations, especially carbon dioxide and water, together with ammonium compounds or nitrates, and a few mineral substances, which serve as its food, the plant builds up the extremely complicated constituents of its organism, proteids, carbohydrates, fats, resins, organic acids, etc. The chemical work which is performed in the plant must therefore, in the majority of cases, consist in syntheses; but besides these, processes of reduction take place to a great extent. The *vis viva* of the sunlight induces the green parts of the plant to split off oxygen from the carbon dioxide and water, and therefore the chief constituents of the plant contain less oxygen than the material serving as food. The *vis viva* of the sun, which produces this splitting, is not lost; it is only transformed into another form of force—into the potential energy or chemical tension of the free oxygen on the one side, and the combinations less oxygenated, produced by the synthesis, on the other side.

These conditions are not the same in animals. They are dependent either directly, as the herbivora, or indirectly, as the carnivora, upon plant-life, from which they derive the three chief groups of organic nutritive matter—proteids, carbohydrates, and fats. These bodies, of which the protein substances and fat form the chief mass of the animal body, undergo within the animal organism a splitting and oxidation, and yield as final products exactly the above-mentioned chief components of the nutrition of plants, namely, carbon dioxide, water, and ammonia derivatives, which are rich in oxygen and have feeble potential energy. The chemical tension, which is partly combined with the free oxygen and partly stored up in the above-mentioned more complex chemical compounds, is transformed into *vis viva*, heat, and mechanical work. While in the plant reduction processes and syntheses, which are active in the conversion of living force into potential energy or chemical tension, are the prevailing forces, we find in the animal body the reverse of this, namely, splitting and oxidation processes, which convert chemical tension into living force (*vis viva*).

This difference between animals and plants must not be overrated, nor must we consider that there exists a sharp boundary-line between the two. This is not the case. There are not only lower plants, free from chlorophyll, which in regard to chemical processes represent intermediate steps between higher plants and animals, but the difference existing between the higher plants and animals is more of a quantitative than a qualitative kind. Plants require oxygen as peremptorily as do animals. Like the animal, the plant also, in the dark and by means of those parts which are free from chlorophyll, takes up oxygen and eliminates carbon dioxide, while in the light the oxidation processes going on in the green parts are overshadowed or hidden beneath the more intense reduction processes. Like the animal the fermentive fungi transform chemical tension into living energy and heat; and even in a few of the higher plants—as the *aroideæ* when bearing fruit—a considerable development of heat has been observed. The reverse is found in the animal organism, for, besides oxidation and splitting, reduction processes and syntheses also take place. The contrast which seemingly exists between animals and plants consists merely in that in the animal organism the processes of oxidation and splitting are prevalent, while in the plant those of reduction and synthesis have thus far been observed.

WÖHLER[1] in 1824 furnished the first example of SYNTHETICAL PROCESSES within the animal organism. He showed that when benzoic acid is introduced into the stomach it reappears as hippuric acid in the urine, after it combines with glycocoll (amido-acetic acid). Since the discovery of this synthesis, which may be expressed by the following equation,

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[1] Berzelius, Lehrb. d. Chemie, übersetzt von Wöhler, Bd. 4. Dresden, 1831. S. 376, Anm.

$$\underset{\text{Benzoic acid}}{C_6H_5.COOH}+\underset{\text{Glycocoll}}{NH_2.CH_2.COOH}=\underset{\text{Hippuric acid}}{NH(C_6H_5.CO).CH_2.COOH}+H_2O,$$

and which is ordinarily considered as a type of an entire series of syntheses occurring in the body where water is eliminated, the number of known syntheses in the animal kingdom has increased considerably. Many of these syntheses have also been artificially produced outside of the organism, and numerous examples of animal syntheses of which the course is absolutely clear will be found in the following pages. Besides these well-studied syntheses, there occur in the animal body also similar processes unquestionably of the greatest importance to animal life, but of which we know nothing with positiveness. We enumerate as examples of this kind of synthesis the reformation of the red-blood pigment (the hæmoglobin), the formation of the different proteids from the peptones, the formation of fat from carbohydrates, and others.

The chemical processes in the animal body we have mentioned above as consisting chiefly of oxidation and splitting processes. The oxygen of inhaled air, as also that of the blood, is now called neutral, molecular oxygen, and the old assumption that ozone occurs in the organism has now been discarded for several reasons. There are but few substances which can be oxidized within the animal organism by the neutral oxygen; while, on the contrary, proteids and fat, which form the chief part of the organic constituents of the animal body, are almost indifferent to neutral oxygen. The question arises, how then is the oxidation of these and other bodies possible in the animal organism?

Formerly the view was generally accepted that ANIMAL OXIDATION took place in the fluids, while to-day we are of the opinion, derived from the investigations of PFLÜGER and his pupils,[1] that it is connected with the form-elements and the tissues. The question how this oxidation in the form-elements proceeds and how it is induced cannot be answered with certainty.

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[1] Pflüger, Pflüger's Archiv, Bdd. 6 and 10; Finkler, *ibid.*, Bdd. 10 and 14; Oertman, *ibid.*, Bdd. 14 and 15; Hoppe-Seyler, *ibid.*, Bd. 7.

The cause of the animal oxidation is considered, by PFLÜGER and several other investigators, to be dependent upon the special contitution of the protoplasmic proteids. This investigator calls the proteids outside of the organism, and also those which circulate in the blood and fluids, "non-living proteids" as compared to those which are converted by the activity of the living cell into living protoplasm, which he calls "living proteids." It is now also considered that this "living proteid" differs from the "non-living proteid" by a greater mobility of the atoms within the molecule, and it may be characterized by a greater inclination towards intramolecular changes of position of these atoms.

The reason for these greater intramolecular movements PFLÜGER[1] ascribes to the presence of cyanogen, LOEW[2] to the presence of aldehydic groups, and LATHAM[3] attributes it to the presence of a chain of cyanalcohols in the proteid molecule.

PFLÜGER considers these differences between ordinary proteids and living protoplasmic proteids as the cause for the oxidation processes in the animal organism. These processes show certain similarity to the oxidation of phosphorus in an atmosphere containing oxygen. In this process the phosphorus is not only itself oxidized, but, as it splits the oxygen molecules and sets free oxygen atoms (active oxygen), it may cause at the same time an indirect or secondary oxidizing action upon other bodies present. In an analogous way the living protoplasmic proteid, which is not, like dead proteid, indifferent to molecular oxygen, may cause a splitting of the oxygen molecule, thus becoming itself oxidized, and at the same time setting oxygen atoms free, which may cause a secondary oxidation of other less oxidizable substances.

Active oxygen may also be produced, according to O. NASSE,[4] by a hydroxylization of the constituents of the protoplasm with the splitting off of molecules of water. If benzaldehyde is shaken with water and air an oxidation of the benzaldehyde into benzoic acid takes place, while oxidizable substances present at the same time may also be oxidized. The simultaneous presence of potassium iodide and starch or tincture of guaiacum causes a blue coloration because the hydroxyl (OH) takes the place of the hydrogen in the aldehyde group and these two hydrogen atoms, one derived from the aldehyde and the other from the splitting of the water, have a splitting action on the molecular oxygen. Nasse and Rösing[1] have found that certain varieties of proteid have the property of being hydroxylized in the presence of water, and a series of oxidations in the animal body may, according to Nasse, be accounted for by the oxygen atoms set free in the hydroxylization similar to that of benzaldehyde.

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[1] Pflüger's Archiv, Bd. 10.

[2] Loew and Bokorny, Pflüger's Archiv, Bd. 25, and Loew, *ibid.*, Bd. 30.

[3] British Medical Journal, 1886.

[4] Rostocker Zeitung, 1891, No. 534.

Another very widely diffused view exists in regard to the origin of the activity of the oxygen, namely, that by the decomposition processes in the tissues reducing substances are formed which split the oxygen molecule, uniting with one oxygen atom and setting the other free.

The formation of reducing substances during fermentation and putrefaction is generally known. The butyric fermentation of dextrose in which hydrogen is set free—$C_6H_{12}O_6 = C_4H_8O_2 + 2CO_2 + 2(H_2)$—is an example of this kind. Another example is the appearance of nitrates in consequence of an oxidation of nitrogen in cases of putrefaction, which process is ordinarily explained by the statement that, in putrefaction, reducing, easily oxidizable bodies are formed which split oxygen molecules, liberating oxygen atoms which afterward oxidize the nitrogen. It is assumed also that the cells of the animal tissues and organs have the property like these lower organisms, which cause fermentation and putrefaction, of causing splitting processes in which easily oxidizable substances, perhaps also hydrogen *in statu nascendi* (Hoppe-Seyler[2]), are produced. The observations of Ehrlich,[3] that certain blue coloring matters—alizarin blue and indophenol blue—are decolorized by the tissues of the living animal and become blue again on exposure to air, seem also to be a proof of the occurrence of easily oxidizable combinations in the tissues. A further proof of this is found in the observations of C. Ludwig and Alex. Schmidt[4] that in the blood of asphyxiated animals, as well as in the absence of oxygen, an accumulation of reducing, easily oxidizable substances takes place.

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[1] Ernst Rösing, Untersuchungen über die Oxydation von Eiweiss in Gegenwart von Schwefel. Inaug. Dissert. Rostock, 1881.

[2] Pflüger's Archiv, Bd 12.

[3] P. Ehrlich, Das Sauerstoffbedürfniss des Organismus. Berlin, 1885.

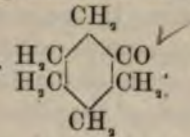
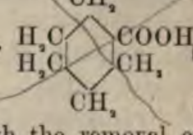
[4] Arbeiten aus der physiol. Anstalt zu Leipzig. 1867.

In accordance with what has been stated above, we may assume that the oxidation in the animal body takes place in the following manner: The forces peculiar to protoplasm, unknown to us, but acting similarly to heat or the enzymes, cause a splitting, producing reducible and readily oxidizable products on one side and difficultly oxidizable products on the other. The first may be directly oxidized, and as they cause a splitting of the molecular oxygen, setting active oxygen free, they may also be the indirect cause of the oxidation of the more difficultly oxidizable substances, namely causeing a SECONDARY OXIDATION.[1] The products formed by these splittings and oxidations may perhaps in part be burned within the body without undergoing further splitting, but they must probably first undergo a further splitting and then succumb to consecutive oxidation, until after repeated splitting and oxidation the final products of metabolism are formed.

The oxidations in the animal body have long been designated as a combustion, and such a view is easily reconcilable with the abovementioned views. In combustion in the ordinary sense, as, for example, the burning of wood or oil, we must not forget that the substances themselves do not combine with oxygen. It is only after the action of heat has decomposed these bodies to a certain degree that the oxidation of the products of such decomposition takes place and is accompanied by the phenomenon of light.

The numerous intermediary products of decomposition which we observe in the animal body teach us that the oxidations and splittings of the components of the body do not take place at once and suddenly, but only very gradually, step by step, until the final products of exchange are reached.

A very instructive example of such a gradual decomposition outside of the organism has been shown by DRECHSEL[2] in his investigation on the electrolysis of phenol by an alternating current. By experiments with alternating electric currents we obtain, of course, in the watery solution of the substance, at each electrode alternately, oxygen and hydrogen in great rapidity. Therefore oxidations and reductions must take place alternately, and we obtain syntheses as well as splittings with oxidations.

If phenol in watery solution is exposed to such an alternating current, we produce, by the combined action of reduction and oxidation processes, a new body—hydro-phenoketon, $C_6H_{10}O$—by aggregation of hydrogen atoms with the simultaneous rupture of all double bonds of the benzol ring and then an oxidation with the removal of hydrogen atoms or . From the hydrophenoketon a compound of the fatty series is produced by the fixation of O + 2H accompanied with the splitting of the benzol ring, namely, normal caproic acid, $C_6H_{12}O_2$, or 

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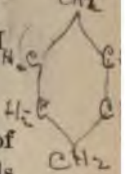
[1] O. Nasse, Pflüger's Archiv, Bd. 41.

[2] Journal f. prakt. Chemie (N. F.), Bd. 22, 29, 38; also Festschrift f. C. Ludwig, 1887.



By further electrolysis of the caproic acid, with the removal of carbon as carbon dioxide and of hydrogen as water, a series of acids with decreasing amounts of carbon are obtained, and in this way we may, by properly directed combination of reductions and oxidations, pass from a body of the aromatic series to a body of the fatty series, and then to substances in which the amount of carbon decreases, until the final metabolic products are reached.

As Drechsel has also found that the same electro-syntheses (of urea and phenol-sulphuric acid) are produced by the continuous as with the alternating current, and since the occurrence of galvanic currents in the body has been positively shown, Drechsel concludes that not only do syntheses, but also the combustion of foods and constituents of the tissues, take place in the animal body in consequence of a quick succession of reductions and oxidations produced in this way.

ome

Most investigators are without doubt agreed in the view that a united action of oxidation and reduction processes takes place in the animal body. The views in regard to the kind and origin of this co-operative action are divided.[1]

In the previous pages we have spoken of the formation of active oxygen, but there are also investigators who do not assent to such a theory, or at least not entirely. Traube[2] has brought forward powerful arguments against the view that the so-called slow combustion or spontaneous oxidation causes a splitting of the oxygen molecule. He has shown that this theory does not account for many cases of auto-oxidation. TRAUBE[1] for a long time has explained the oxidations in the animal organism by the statement that within the organism so-called oxygen-carriers occur which act similarly to nitric oxide in the sulphuric-acid manufacture, where oxidation is the result of the absorption and liberation of oxygen by other substances which are themselves not directly oxidized by molecular oxygen.

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[1] M. Nencki, Arch. des sciences biol. de l'Institut impérial de Médecine expér. à St. Petersbourg. Tome 1, No. 4, p. 483.

[2] Ber. d. deutsch. chem. Gesellsch., Bdd. 15, 18, 19, and 26.

DE REY-PAILHADE[2] has been able to isolate such a body from yeast and animal tissues. He calls the body *philothion*, and it has the property of developing sulphuretted hydrogen from finely divided sulphur. This substance, which seems to be a combination of hydrogen with a hypothetical radicle, can take up oxygen and form water. The radicle set free takes up hydrogen from water by splitting, setting free oxygen, which acts upon other bodies, oxidizing them. The regenerated philothion takes up oxygen again, and so the processes go on. NASSE and RÖSING[3] explain the observations of DE REY-PAILHADE in another way.

The observation first made by JAQUET[4] and then positively confirmed by SALKOWSKI,[5] SPITZER,[6] ABELOUS, and BIARNÈS[7] that a body similar to a ferment occurs in various tissues and also in the blood, which has the property of oxidizing certain bodies such as benzalcohol, salicylic aldehyde, and dextrose. Nothing positive can be given at the present time as to the importance of this *oxidation ferment* in the oxidation in the animal body.

ROHMANN[8] and SPITZER[9] have shown that there exist oxidation ferments in the cells and tissues of the body which act as oxygen-carriers in TRAUBE's sense. These bodies, whose activity is destroyed by heat, not only have an action on hydrogen peroxide but also on neutral oxygen, which the authors have shown by special pigment syntheses. Thus the syntheses of indophenol from α-naphthol and paraphenylendiamin only take place gradually in the air in the presence of alkali, whilst a very small quantity of fresh organ pulp causes an action in a few minutes. The oxidation of the dextrose in the blood, the so-called glycolysis, is also produced by oxygen-carriers. The authors are therefore not of the opinion that all oxidations of difficultly combustible bodies in the organism are caused by the oxygen-carriers. The oxygen-carriers are not identical with the auto-oxidizable bodies; not those as considered by HOPPE-SEYLER as the cause of oxidation, but those which always act reducing.

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[1] Traube, Theorie der Fermentwirkungen. Berlin, 1858.

[2] Recherches exper. sur le Philothion, etc. Paris, 1891, and Nouvelles recherches sur le Philothion. Paris, 1892.

[3] Unters. über die Oxydation von Eiweiss in Gegenwart von Schwefel. Inaug. Dissert. Rostock, 1891.

[4] Arch. f. expt. Path. u. Pharm., Bd. 29.

[5] Centralbl. f. d. med. Wissensch., 1892 and 1894.

[6] Berlin. klin. Wochenschr., 1894.

[7] Arch. de Physiol. (5), Tome 6.

[8] Ber. d. deutsch. chem. Gesellsch., Bd. 28.

[9] Pflüger's Arch., Bd. 60.

An important source of the living energy developed in the body is to be sought for in the oxidation effected by oxygen of strong potential energy, but SPLITTING PROCESSES are also important. In these complicated chemical compounds are reduced to simpler ones, and therefore the atoms change from a mobile equilibrium to a stabler one and stronger chemical affinities are satisfied, converting chemical potential energy into living energy (*vis viva*). The best-known example of such a splitting process outside of the animal organism is the ordinary alcoholic fermentation of dextrose, $C_6H_{12}O_6 = 2CO_2 + 2C_2H_6O$, in which process heat is set free. The animal body may also have a source of energy in the splitting processes which are not dependent on the presence of free oxygen. The processes taking place in the living muscle yield an example of this kind. A removed muscle, which gives no oxygen when in a vacuum, may, as HERMANN[1] has shown, work, at least for a time, in an atmosphere devoid of oxygen, and give off carbon dioxide at the same time.

We call processes of splitting which are accompanied by a decomposition of water and then a taking up of its constituents *hydrolytic splittings*. These splittings, which play an important rôle within the animal body, and which are most frequently met with in the process of digestion, are, for example, the transformation of starch into dextrose and the splitting of neutral fats into the corresponding fatty acid and glycerin:

$$\underset{\text{Tristearin.}}{C_3H_5(C_{18}H_{35}O_2)_3} + 3H_2O = \underset{\text{Glycerin.}}{C_3H_5(OH)_3} + \underset{\text{Stearic acid.}}{3(C_{18}H_{36}O_2)}.$$

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[1] Untersuchungen über den Stoffwechsel der Muskeln. Berlin, 1867.

As a rule the hydrolytic splitting processes as they occur in the animal body may be performed outside of it by means of higher temperatures with or without the simultaneous action of acids or alkalies. Considering the two above-mentioned examples, we know that starch is converted into dextrose when it is boiled with dilute acids, and also that the fats are split into fatty acids and glycerin on heating them with caustic alkalies or by the action of superheated steam. The heat or the chemical reagents which are used for the performance of these reactions would cause immediate death if applied to the living system. Consequently the animal organism must have other means at its disposal which act similarly, but in such a manner that they may work without endangering the life or normal constitution of the tissues. Such means have been recognized in the so-called *unorganized ferments* or *enzymes*.

Alcoholic fermentation, as well as other processes of fermentation and putrefaction, is dependent upon the presence of living organisms, ferment fungi and splitting fungi of different kinds. The ordinary view, according to the researches of PASTEUR, is that these processes are to be considered as phases of life of these organisms. The name *organized ferments* or *ferments* has been given to such micro-organisms of which ordinary yeast is an example. However, the same name has also been given to certain bodies or mixtures of bodies of unknown organic origin which are products of the chemical work within the cell, and which, after they are separated from the cell, are capable in the smallest quantities of causing a decomposition or splitting in very considerable quantities of other substances without entering into combination with the decomposed body or with any of its products of splitting or decomposition. Such ferments are, for example, the diastase of malt and the ferments secreted by the different glands participating in the process of digestion. These formless or *unorganized ferments* are generally called, according to KÜHNE, *enzymes*.

A ferment in a more restricted sense is therefore a living being, while an enzyme is a product of chemical processes in the cell, a product which has an individuality even without the cell, and which may be active when separated from the cell. The splitting of invert-sugar into carbon dioxide and alcohol by fermentation is a fermentative process closely connected with the life of the yeast. The inversion of cane-sugar is, on the contrary, an enzymotic process caused by one of the bodies or mixture of bodies formed by the living ferment, which can be severed from this ferment, and still remains active even after the death of the latter. Consequently ferments and enzymes are capable of manifesting a different behavior towards certain chemical reagents. Thus there exist a number of substances, among which we may mention arsenious acid, phenol, salicylic acid, boracic acid, chloroform, ether, and others, which in certain concentration kill ferments, but which do not noticeably impair the action of the enzymes. A very serviceable substance in this regard is, according to the investigations of ARTHUS and HUBER,[1] a 1% solution of sodium fluoride.

The enzymes may as above stated act when separated from the cell, and are thus extracellular, but this does not preclude the possibility that we also may have enzymes which develop their action within the cell and therefore are intracellular. As an example of such an enzyme we may mention the enzyme existing in the micrococcus ureæ which has the power of decomposing urea, and also another enzyme, produced by a bacterium, which decomposes calcium formate into calcium carbonate and hydrogen.

It is doubtful, indeed highly improbable, whether it has been possible up to the present time to isolate any enzyme in a pure state. Therefore the nature of the enzymes and their elementary composition are unknown. Such as have been obtained thus far appear to be nitrogenized and to be similar in some degree to proteid bodies. The enzymes are considered as proteid bodies by many investigators, but this opinion has not sufficient foundation. It is indeed true that the enzymes isolated by certain investigators act like genuine proteid bodies; but it is undecided whether or not the products isolated in these instances were pure enzymes or were composed of enzymes contaminated with proteids.

The enzymes may be extracted from the tissues by means of water or glycerin, especially by the latter, which forms very stable solutions and consequently serves as a means of extracting them. The enzymes, generally speaking, do not appear to be diffusible. They are readily carried down with other substances when these precipitate in a finely divided state, and this property is extensively taken advantage of in the preparation of pure enzymes.[2]

The property of many enzymes of decomposing hydrogen peroxide is, according to ALEX. SCHMIDT,[1] not dependent upon the enzyme, but is caused by the contamination of the enzyme with constituents from the protoplasm. This coincides with the observations of JACOBSON[2] on emulsin, pancreas enzyme, and diastase that the catalytic property may be destroyed by proper means without diminishing the specific enzymotic action. The continued heating of their solutions above + 80° C. generally destroys most of the enzymes. In the dry state, however, certain enzymes may be heated to 100° or indeed to 150°-160° C. without losing their power. The enzymes are precipitated from their solutions by alcohol.

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[1] Archives de Physiologie, 1892. (5) Tome 4.

[2] Brücke, Wiener Sitzungsbericht, Bd. 43. 1861.

We have no characteristic reactions for the enzymes in general, and each enzyme is characterized by its specific action and by the conditions under which it operates. But it must be stated that, however the different enzymes may vary in action, they all seem to have this in common, that by their presence an impulse is given to split more complicated combinations into simpler ones, whereby the atoms arrange themselves from an unstable equilibrium into a more stable one, chemical tension is transformed into living force, and new products are formed with lower heat of combustion than the original substance. The presence of water seems to be a necessary factor in the perfection of such decompositions, and the chemical process seems to consist in the taking up of the elements of water.

The action of the enzymes may be markedly influenced by external conditions. The reaction of the liquid is of special importance. Certain enzymes act only in acid, others, and the majority, on the contrary act only in neutral or alkaline liquids. Certain of them act in very faintly acid as well as in neutral or alkaline solutions, but best at a specific reaction. The temperature exercises also a very important influence. In general the activity of enzymes increases to a certain limit with the temperature. This limit is not always the same, but is dependent upon the quantity of enzyme.[3] The products of the enzymotic processes exercise a retarding influence. Additions of various kinds may have a retarding and others an accelerating action.

FERMI and PERNOSSI[4] have studied the action of various influences on the enzymes. Starting with the assumption that when the free ions are set free by the action of enzymes the electrical conductivity of the water must be raised, O. NASSE[1] experimented with soluble starch, partly boiled and partly unboiled, and diastase, and determined the resistance according to KOHLRAUSCH'S method and observed a considerable increase in the conductivity of the active diastase solutions.

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[1] Al. Schmidt, Zur Blutlehre. Leipzig, 1892.

[2] Zeitschr. f. physiol. Chemie, Bd. 16, S. 340.

[3] Tammann, Zeitschr. f. physiol. Chem., Bd. 16, S. 271.

[4] Zeitschr. f. Hygiene, Bd. 18.

The animal enzymes are divided into several groups. The most studied of these are the hydrolytic enzymes found in the digestive canal. The three most important groups are the *amylolytic* or diastatic, the *proteolytic* or those converting proteids into soluble modifications, and the *steatolytic* or fat-splitting enzymes. The *coagulating* enzymes form a peculiar group. The mode of action of these enzymes, amongst which we reckon chymosin (rennin) or casein-coagulating, and fibrin ferment or blood-coagulating, is still less known than the others. The manner in which these enzymes work is still obscure, but their action may be considered, in several respects, as very closely related to the so-called catalytic or contact action.

As above stated, the enzymes are of great importance for the chemical processes going on in the digestive tract, but we have to add that the results of their action are greatly complicated by processes of putrefaction which take place in the intestine at the same time, and which are caused by micro-organisms. Micro-organisms therefore exercise a certain influence on the physiological processes of the animal body. These organisms, when they enter the animal fluids and tissues and develop and increase, are of the greatest pathological importance, and modern bacteriology in relation to the doctrine of infectious diseases, founded by PASTEUR and KOCH, gives efficient testimony to these facts.

Putrefaction caused within the animal fluids and tissues by lower organisms may produce, among others, combinations of a basic nature. Such bodies were first found by SELMI in human cadavers, and called by him cadaver alkaloids or *ptomaines*. These ptomaines, which have been isolated from cadavers and some from putrefying proteid mixtures, have been closely studied by SELMI,[2]

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[1] Rostocker Zeitung, 1894.

[2] Sulle ptomaine od alcaloidi cadaverici e loro importanza in tossicologia. Bologna, 1878. Ber. d. deutsch. chem. Gesellsch., Bd. 11.

BRIEGER,[1] and GAUTIER,[2] and are considered as products of chemical processes caused by putrefaction microbes. The first ptomaine to be analyzed was *collidin*, $C_8H_{11}N$, obtained by NENCKI,[3] on the putrefaction of gelatin. Since then many ptomaines have been anlayzed by GAUTIER, and especially by BRIEGER. Certain of the ptomaines originate undoubtedly from lecithin and other so-called extractives of the tissues, but the majority seem to be derived from the protein substances by decomposition.

Some ptomaines, although all belong to the aliphatic series, contain oxygen and others are free from oxygen. The majority of the true ptomaines belong to the latter group. Most of the ptomaines isolated by BRIEGER are diamines or compounds derived from the same. Amongst the diamines we have two, *cadaverin* or pentamethylendiamin, $C_5H_{14}N_2$, and *putrescin* or tetramethylendiamin, $C_4H_{12}N_2$, which are of special interest because they have been found in the intestinal tract and urine in certain pathological conditions, namely, cholera[4] and cystinuria.[5] Some of the ptomaines are exceedingly poisonous, while others are not. The poisonous ones are called *toxines*, according to the suggestion of BRIEGER.

The formation of such toxines in the decompositions caused by putrefactive microbes makes it probable that the lower organisms acting in infectious diseases also produce poisonous substances which may cause by their action the symptoms or complications of the disease. BRIEGER, who has become prominent by his study of this subject, has been able to isolate from typhus cultures a substance called *typhotoxin* which has a poisonous action on animals; and he has also prepared another substance, *tetanin*,[6] from the amputated arm of a patient with tetanus, animals inoculated with which die exhibiting symptoms of developed tetanus.

As above stated, the chemical processes in animals and plants do not stand in opposition to each other; they offer differences indeed, but still they are of the same kind from a qualitative standpoint.

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[1] Ueber Ptomaine, Parts 1, 2, and 3. Berlin, 1885–1886.

[2] Traité de chimie appliquée à la physiologie, Tome 2, 1873. Compt. rendus, Tome 94.

[3] Ueber die Zersetzung der Gelatine, etc. Bern, 1876.

[4] Brieger, Berlin. klin. Wochenschr., 1887.

[5] Baumann and Udransky, Zeitschr. f. physiol. Chem., Bdd. 13 and 15; Brieger and Stadthagen, Berlin. klin. Wochenschr., 1889.

[6] Brieger, Arch. f. pathol. Anat., Bdd. 112 and 115. Also Sitzungsber. d. Berl. Akad. d. W., 1889, and Berl. klin. Wochenschr., 1888.

Pflüger says that there exists a blood-relationship between all living cells of the animal and vegetable kingdoms, and that they originate from the same root; and if the organisms consisting of one cell can decompose protein substances in such a manner as to produce poisonous substances, why should not the animal body, which is only a collection of cells, be able to produce under physiological conditions similar poisonous substances? It has been known for a long time that the animal body possesses this ability to a great extent, and as well-known evidence of this ability we may mention various nitrogenized extractives and poisonous constituents of the secretions of certain animals. Those substances of basic nature which are incessantly and regularly produced as products of the decomposition of the protein substances in the living organism, and which therefore are to be considered as products of the physiological exchange of material, have been called *leucomaines* by **Gautier**[1] in contradistinction to the ptomaines and toxines produced by micro-organisms. These bodies, to which belong several well-known animal extractives, were isolated by **Gautier** from animal tissues such as the muscles. The hitherto known leucomaines, of which a few are poisonous in small amounts, belong to the cholin, the uric acid, and the creatinin group.

The leucomaines are considered as being of certain importance as causes of disease. It has been contended that when these bodies accumulate on account of an incomplete excretion or oxidation in the system, an auto-intoxication may be produced (**Bouchard**[2]).

The toxines and the poisonous leucomaines are, however, neither the only nor the most active poison produced by the plant or animal cell. Later investigations have shown that certain plants as well as animals can produce proteids which are exceedingly poisonous. Such poisonous proteids have, for example, been isolated from the jequirity and castor beans, as also from the venom of snakes, spiders, and other animals. The toxic proteids produced by pathogenic micro-organisms are of special interest. Proteids have been isolated from the cultures of various pathogenic microbes within the last few years (BRIEGER and FRÄNKEL[1]) which are exceedingly poisonous, and which reproduce the symptoms of the infection more exactly than the toxine. These proteids have been called *toxalbumins* by BRIEGER and FRÄNKEL.

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[1] Bull. soc. chim., 43, and A. Gautier, Sur les alcaloïdes derivés de la destruction bactérienne ou physiologique des tissus animaux. Paris, 1886.

[2] Bouchard, Leçons sur les auto-intoxications dans les maladies. Paris, 1887.

It is of great interest that we know also of proteid bodies some of which, like the so-called *alexines* in the blood serum, have a germicidal, or bactericidal action, while others make the animal body *immune* against infection with a certain microbe or protect the body against the poison produced by the microbe. The great importance of these observations is apparent, but as it is not within the range of this book we will not further discuss the subject. The nature of these remarkable proteids will be given somewhat in detail in the following chapter.

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[1] Berl. klin. Wochenschr., 1890.

CHAPTER II.

THE PROTEIN SUBSTANCES.

THE chief mass of the organic constituents of animal tissues consists of amorphous, nitrogenized, very complex bodies of high molecular weight. These bodies, which are either proteids in a special sense or bodies nearly related thereto, take first rank among the organic constituents of the animal body on account of their great abundance. For this reason they are classed together in a special group which has received the name *protein group* (from *πρωτεύο*, I am the first, or take the first place). The bodies belonging to these several groups are called *protein substances*, although in a few cases the proteid bodies in a special sense are designated by the same name.

The several *protein substances* contain *carbon*, *hydrogen*, *nitrogen*, and *oxygen*. The majority contain also *sulphur*, a few *phosphorus*, and a few also *iron*. *Copper* has been found in some few cases. On heating the protein substances they gradually decompose, producing inflammable gases, ammoniacal compounds, carbon dioxide, water, nitrogenized bases, as well as many other bodies, and at the same time they emit a strong odor of burnt horn or wool. More highly heated they leave a porous, shining mass of carbon, and when this is thoroughly burnt an ash is obtained consisting chiefly of calcium and magnesium phosphates. The question whether the mineral bodies left by burning exist as impurities or whether they are constituents of the protein molecule has not been decided.

It is at present impossible to decide on a classification of the protein substances based upon their properties, reactions, and constitution, as well as upon their solubilities and precipitations, corresponding to the demands of science. The best classification is perhaps the following systematic summary of the better known and studied animal protein substances, due chiefly to HOPPE-SEYLER and DRECHSEL.[1]

I. Simple Proteids or Albuminous Bodies.

Albumins	*Seralbumin,* *Ovalbumin,* *Lactalbumin.*
Globulins	*Serglobulin,* *Fibrinogen,* *Myosin,* *Musculin,* *Crystallin,* *Vitellins* (*?*).
Nucleo-albumins...	*Casein,* *Ovovitellin* (*?*), and others.
Albuminates	*Acid albuminate,* *Alkali albuminate.*
Albumoses and **Peptones.**	
Coagulated Proteids	*Fibrin,* Proteids coagulated by heat, and others.

II. Compound Proteids.

Hæmoglobins.	
Glycoproteids	*Mucins* and *Mucinoids,* *Hyalogens,* *Ichthulin,* *Helicoproteid.*
Nucleoproteids	*Nucleohiston,* *Cytoglobin,* and others.

III. Albumoids or Albuminoids.

Keratin.
Elastin.
Collagen.
Reticulin.
(Amyloid.)
(Fibroin, Sericin, Cornein, Spongin, Conchiolin, Byssus, and others.)

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[1] See "Eiweisskörper," Ladenburg's Handwörterbuch der Chemie, Bd. 3, S. 534–589.

To this summary must be added that we often find in the investigations of animal fluids and tissues protein substances which do not coincide with the above scheme, or do so only with difficulty. At the same time it must be remarked that bodies will be found which seem to rank between the different groups, hence it is very difficult to sharply divide these groups.

I. Simple Proteids or Albuminous Bodies.

The simple proteids are never-failing constituents of the animal and vegetable organisms. They are especially found in the animal body, where they form the solid constituents of the muscles, glands, and the blood serum, and they are so generally distributed that there are only a few animal secretions and excretions, such as the tears, perspiration, and perhaps urine, in which they are entirely absent or only occur as traces.

All albuminous bodies contain *carbon*, *hydrogen*, *nitrogen*, *oxygen*, and *sulphur*;[1] a few contain also *phosphorus*. *Iron* is generally found in traces in their ash, and it seems to be a regular constituent of a certain group of the albuminous bodies, namely, the nucleo-albumins. The composition of the different albuminous bodies varies a little, but the variations are within relatively close limits. For the better studied animal proteids the following composition of the ash-free substance has been given:

C	50.6 — 54.5	per cent.
H	6.5 — 7.3	"
N	15.0 — 17.6	"
S	0.3 — 2.2	"
P	0.42 — 0.85	"
O	21.50 — 23.50	"

A part of the nitrogen of the proteid molecule is loosely combined and splits off easily as ammonia by the action of alkalies (NASSE[2]). Sulphur shows the same property in nearly all albuminous bodies (Fleitmann,[1] Danilewsky,[2] Krüger[3]). A part of the sulphur separates as potassium or sodium sulphide on boiling with caustic potash or soda, and may be detected by lead acetate. What remains can only be detected after fusing with nitre and sodium carbonate and testing for sulphates. The proteid molecule therefore contains at least 2 atoms of sulphur. The molecular weight of the proteids has not been determined with accuracy up to the present time, therefore it is impossible to give them formulæ. The molecular weight of ovalbumin as determined by Sabanejew and Alexandrow[4] is about 14.300. For the alkali albuminate, in whose formation from native albumins a part of the nitrogen and the loosely bound sulphur is split off, Lieberkühn has given the formula $C_{72}H_{112}N_{18}SO_{22}$.

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[1] An exception is found in the mycoprotein of putrefaction bacteria and the anthraxprotein of the anthrax bacillus, which are sulphur-free proteids. See Nencki and Schaffer, Journ. f. prakt. Chem., Bd. 20 (N. F.), and Nencki, Ber. d. deutsch. chem. Gesellsch., Bd. 17.

[2] Pflüger's Archiv, Bd. 6.

The constitution of the proteid bodies, notwithstanding numerous investigations, is still unknown. By heating proteids with barium hydrate and water in sealed tubes at 150°-200° C. for several days, Schützenberger[5] obtained a number of products among which were ammonia, carbon dioxide, oxalic acid, acetic acid, and, as chief product, a mixture of amido-acids. This mixture contained, besides a little tyrosin and a few other bodies, chiefly acids of the series $C_nH_{2n+1}NO_2$ (*leucines*) and $C_nH_{2n-1}NO_2$ (*leuceines*). The leucines and leuceines are formed from more complicated substances, with the general formula $C_mH_{2m}N_2O_4$, by hydrolytic splitting. These substances are called *glucoproteins* by Schützenberger on account of their sweet taste. The sulphur of the proteids yields sulphites. The three bodies, carbon dioxide, oxalic acid, and ammonia, are formed in the same relative proportion as in the decomposition of urea and oxamid; therefore Schützenberger suggests that perhaps albumin may be considered as a very complex ureid or oxamid. Such a conclusion cannot be derived from the above decomposition processes for several reasons, and the attempts to prepare urea directly by oxidation have also given negative results.

On fusing proteids with caustic alkali, ammonia, mercaptan, and other volatile products are generated; also leucin, from which volatile fatty acids, such as acetic acid, valerianic acid, and also butyric acid, are formed; and tyrosin, from which phenol, indol, and skatol are produced. On boiling with mineral acids, or still better by boiling with hydrochloric acid and tin chloride (Hlasiwetz and Habermann[1]), the proteids yield amido-acids, such as leucin, aspartic acid, glutamic acid, and tyrosin (and from vegetable albumin Schulze and Barbieri[2] obtained α-phenylamidopropionic acid), also sulphuretted hydrogen, ammonia, and nitrogenized bases (Drechsel[3]). As an essential difference between the action of acids and alkalies (barium hydrate) on albumins, Drechsel suggests that by the action of acids carbon dioxide, oxalic and acetic acids are not produced.

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[1] Annal. der Chem und Pharm., Bd. 66.

[2] Zeitschr. f. physiol. Chem., Bd. 7.

[3] Pflüger's Archiv, Bd. 43.

[4] See Maly's Jahresber., Bd. 21, S. 11.

[5] Annal. de Chim. et Phys. (5), 16, and Bull. soc. chim , 23 and 24.

Amongst the bases obtained by Drechsel from casein and by his pupils E. Fischer and M. Siegfried[4] from other proteids and gelatine on boiling with hydrochloric acid and tin chloride, we have one having the formula $C_6H_{13}N_3O_2$ or $C_6H_{11}N_3O + H_2O$, which seems to be homologous to creatin or creatinin and called *lysatin* or *lysatinin* by Drechsel. On boiling lysatinin with baryta-water it yields urea amongst other cleavage products, and it is therefore possible to prepare urea artificially from albumin, without oxidation, by the hydrolysis of this base. Another substance, called *lysin*, has the formula $C_6H_{14}N_2O_2$. From its formula we find that it is homologous with *ornithin*, $C_5H_{12}N_2O_2$ (Jaffé), which it resembles in certain respects (see Chapter XV). Lysin, which is probably diamidocaproic acid, and lysatinin have been shown by Drechsel and Hedin to be produced in the tryptic digestion of fibrin. Drechsel[5] also found diamidoacetic acid amongst the cleavage products of casein.

Proteids are decomposed by the action of proteolytic enzymes in the presence of water. First proteid bodies of lower molecular weight are formed—albumoses and peptones—and then on further decomposition amido-acids such as leucin, tyrosin, and aspartic acid. Both lysin and lysatinin may be produced on far-reaching decomposition (in tryptic digestion). On the extensive decomposition a chromogen may also be formed, which gives a violet color with chlorine- or bromine-water. This chromogen, which is formed in all far-reaching decompositions of proteids where leucin and tyrosin are formed, is called *proteinochromogen* by STADELMANN[1] and *tryptophan* by NEUMEISTER.[2] NENCKI[3] considers this chromogen as the mother substance of various animal pigments. NENCKI[4] has found on the addition of bromine to the digestive fluid containing proteinochromogen that at least two different bodies containing different quantities of bromine are produced. Both bodies show, although not obtained quite pure, a close relationship to certain animal pigments in regard to elementary composition. One stands close to hæmatoporphyrin, or bilirubin, and the other to the animal melanins.

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[1] Annal. d. Chem. u. Pharm., Bdd. 159 and 169.

[2] Ber. d deutsch. chem. Gesellsch., Bd. 16.

[3] Sitzungsber. d. math.-phys. Klasse der k. sächs. Gesellsch. d. Wissenschaften. 1889.

[4] Drechsel gives a complete review of his own and his pupils Fischer, Siegfried and Hedin's investigations on this subject in Du Bois-Reymond's Archiv, 1891: "Der Abbau der Eiweissstoffe."

[5] Ber. d. k. sächs. Gesellsch. d. Wissensch., 1892.

A great many substances are produced in the putrefaction of proteids. First the same bodies as are formed in the decomposition by means of proteolytic enzymes are produced, and then a further decomposition occurs with the formation of a large number of bodies belonging to both the alipathic and aromatic series. Belonging to the first series we have ammonium salts of volatile fatty acids, such as caproic, valerianic, and butyric acids, also carbon dioxide, methane, hydrogen, sulphuretted hydrogen, methylmercaptan,[5] and others. The ptomaines also belong to these products and are probably formed by very different chemical processes or even syntheses.

E. SALKOWSKI[6] divides the putrefactive products of the aromatic series into three groups: (*a*) the phenol group, to which tyrosin, the aromatic oxy-acids, phenol, and cresol belong; (*b*) the phenyl group, including phenylacetic acid and phenylpropionic acid; and lastly (*c*) the indol group, which includes indol, skatol, and skatolcarbonic acid. These various aromatic products are formed during the putrefaction with access of air. NENCKI and BOVET[7] obtained only p.-oxyphenylpropionic acid, phenylpropionic acid, and skatolacetic acid on the putrefaction of proteids by anaërobic schizomycetes in the absence of oxygen. These three acids are produced by the action of nascent hydrogen on the corresponding amido-acid, namely, tyrosin, phenylamidopropionic acid, and skatolamidoacetic acid, and these three last-mentioned amido-acids exist, according to NENCKI, preformed in the proteid molecule.

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[1] Zeitschr. f. Biologie, Bd. 26.

[2] *Ibid.*, S. 329.

[3] Schweizerische Wochenschr. f. Pharmacie, 1891.

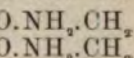
[4] Ber. d. deutsch. chem. Gesellsch., Bd. 28.

[5] See Nencki and Sieber: Monatshefte f. Chem., Bd. 10.

[6] Zeitschr. f. physiol. Chem., Bd. 12, S. 215.

[7] Monatshefte f. Chem., Bd. 10.

On the putrefaction of proteids, as well as their decomposition by means of acids or alkalies and also by certain enzymes, among other products amido-acids are produced, and these have a certain significance for the probable formation of the proteids. It is more than likely that in the synthesis of proteids in the plant from the ammonia or the nitric acid of the soil, amido-acids or acid amids, among which asparagin plays an important rôle, are produced; and from these the albuminous bodies are derived by the influence of glucose or other non-nitrogenized combinations.

Since GRIMAUX[1] was able to prepare by synthetical means from amido-acids bodies which in certain regards were similar to protein substances, so later SCHÜTZENBERGER,[2] by heating a mixture of leucines and leuceines with urea and phosphoric anhydride, obtained a substance which was so similar to peptone in its behavior with several reagents that it was called *pseudopeptone*. The synthetical preparation of protein-like substances by LILIENFELD and WOLKOWICZ[3] in KOSSEL's laboratory is of great importance. The experiments started from the observation of CURTIUS and GOEBEL that amidoacetic acid ethylester readily splits with the separation of a base whose formula is probably  $NH<\begin{matrix}CO.NH_2.CH_2\\CO.NH_2.CH_2\end{matrix}$ according to LILIENFELD and WOLKOWICZ, and that this base or its carbonate, when warmed with water, is transformed into a flocculent body similar to gelatine. This body behaves with reagents and also in regard to elementary composition exactly like gelatine, and its combination with hydrochloric acid has the same composition as glutinpeptone-hydrochloride as prepared by PAAL. On the condensation of other amido-acid esters, namely, the amido-acid ester of leucin and tyrosin with amidoacetic acid elthylester, LILIENFELD and WOLKOWICZ have been able to prepare a substance which, as far as investigated, does not differ in any regard from the peptones or albumoses except in the lack of sulphur. They have also prepared synthetically, by means of a method not completely described, a body which acts like native albumin coagulated by heat.

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[1] Compt. rend., Tome 93, and Bull. de la soc. chim., Tome 42.

[2] Compt. rend., Tome 106 and 112.

[3] Du Bois-Reymond's Arch., 1894, physiol. Abth., S. 383 and 555.

By the oxidation of albumin in acid solutions, volatile fatty acids, their aldehydes, nitriles, ketones, as well as benzoic acid are obtained, also hydrocyanic acid by oxidizing with potassium dichromate and acid. Nitric acid gives various nitro-products, such as xanthoproteic acid (VAN DER PANT'S), trinitroalbumin (LOEW) or oxynitroalbumin, nitrobenzoic acid, and others. With aqua regia fumaric acid, oxalic acid, chlorazol, and other bodies are produced. By the action of bromine under strong pressure a large number of derivatives are obtained, such as bromanil and tribromacetic acid, bromoform, leucin, leucinimid, oxalic acid, tribromamido-benzoic acid, peptone, and bodies similar to humus.

By the dry distillation of albumin we obtain a large number of decomposition products of a disagreeable burnt odor, and a porous glistening mass of carbon containing nitrogen is left as a residue. The products of distillation are partly an alkaline liquid which contains ammonium carbonate and acetate, ammonium sulphide, ammonium cyanide, an inflammable oil and other bodies, and a brown oil which contains hydrocarbons, nitrogenized bases belonging to the aniline and pyridine series, and a number of unknown substances.

It is impossible here to discuss all the products obtained by the action of different reagents on the albumins, but from the above-described decomposition products from proteids it is clear that the products belong in part to the fatty and in part to the aromatic series. Observers are not decided whether one or more aromatic groups exist preformed in the proteid molecule. According to NENCKI the proteids contain three aromatic groups as mentioned above: the tyrosin (oxyphenylamidopropionic acid), the phenylamidopropionic acid, and the skatolamidoacetic acid. MALY[1] considers it not necessary to recognize more than one aromatic group in the proteid molecule.

By the oxidation of albumin by means of potassium permanganate, MALY obtained an acid, oxyprotosulphonic acid, C 51.21; H 6.89; N 14.59; S 1.77; O 25.54, which is not a product of splitting, but an oxidation product in which the group SH is changed into $SO_2.OH$. This acid does not give the proper color reaction with MILLON'S reagent caused by aromatic monohydroxyl derivatives (see below), nor does it yield the ordinary aromatic splitting products of the proteids. Still the aromatic group is not absent, but it seems to be in another binding from that in ordinary albumin. On oxidizing with potassium dichromate and acid this group appears as benzoic acid, and on fusing with alkali benzol is given off.

The animal albuminous bodies are odorless, tasteless, and ordinarily amorphous. The crystalloids (DOTTERPLÄTTCHEN) occurring in the eggs of certain fishes and amphibians do not consist of pure proteids, but of proteids containing large amounts of lecithin, which seems to be combined with mineral substances. Crystalline proteids[1] have been prepared from seeds of various plants, and lately crystallized animal proteids have been prepared by HOFMEISTER.[2] In the dry condition the albuminous bodies appear as a white powder, or when in thin layers as yellowish, hard, transparent plates. A few are soluble in water, others only soluble in salt or faintly alkaline or acid solutions, while others are insoluble in these solvents. All albuminous bodies when burnt leave an ash, and it is therefore questionable whether there exists any proteid body which is soluble in water without the aid of mineral substances. Nevertheless it has not been thus far successfully proved that a native albuminous body can be prepared perfectly free from mineral substances without changing its constitution or its properties.[3] The albuminous bodies are in most cases strong colloids. They diffuse, if at all, only very slightly through animal membranes or parchment-paper, and the proteids therefore have a very high osmotic equivalent. All albuminous bodies are optically active and turn the ray of polarized light to the left.

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[1] Sitzungsber. d. k. Akad. d. Wissensch. Wien, Abth. II, 1885, and Abth. II, 1888. Also Monatshefte f. Chem., Bdd. 6 and 9.

On heating a proteid solution it is changed, the temperature necessary depending upon the proteid present, and with proper reactions of the solution and under favorable external conditions—as, for example, in the presence of neutral salts—most proteids separate in the solid state as "coagulated" proteids. The different temperatures at which various proteids coagulate in neutral salt solutions give in many cases a good means of detecting and separating these various bodies. The views in regard to the use of these means are divided.[4]

The general reactions for the proteids are very numerous, but only the most important will be given here. To facilitate the study of these they have been divided into the two following groups:

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[1] See Maschke, Journ. f. prakt. Chem., Bd. 74; Drechsel, *ibid.* (N. F.), Bd. 19; Grübler, *ibid.* (N. F.), Bd. 23; Ritthausen, *ibid.* (N. F.), Bd. 25; Schmiedeberg, Zeitschr. f. physiol. Chem., Bd. 1; Weyl, *ibid.*, Bd. 1.

[2] Zeitschr. f. physiol. Chem., Bdd. 14 and 16.

[3] See E. Harnack, Ber. d. deutsch. chem. Gesellsch., Bdd. 22, 23, 25; Werigo, Pflüger's Archiv, Bd. 48.

[4] See Halliburton, Journ. of Physiol., Vols. 5 and 11, Corin and Berard, Bull. de l'Acad. roy. de Belg., 15; Haycraft and Duggan, Brit. Med. Journ., 1890, and Proc. Roy. Soc. Ed., 1889; Corin and Ansiaux, Bull. de l'Acad. roy. de Belg., Tome 21; L. Frédericq, Centralbl. f. Physiol., Bd. 3; Haycraft, *ibid.*, Bd. 4; Hewlett, Journ. of Physiol., Vol. 13.

A. Precipitation Reactions of the Proteid Bodies.

1. *Coagulation Test.* An alkaline proteid solution does not coagulate on boiling, a neutral solution only partly and incompletely, and the reaction must therefore be acid for coagulation. The neutral liquid is first boiled and then the proper amount of acid added carefully. A flocculent precipitate is formed, and if properly done the filtrate should be water-clear. If dilute acetic acid be used for this test, the liquid must first be boiled and then 1, 2, or 3 drops of acid added to each 10–15 c.c., depending on the amount of proteid present, and boiled before the addition of each drop. If dilute nitric acid be used, then to 10–15 c.c. of the previously boiled liquid 15–20 drops of the acid must be added. If too little nitric acid be added a soluble combination of the acid and proteid is formed which is precipitated by more acid. A proteid solution containing a small amount of salts must first be treated with about 1% NaCl, since the heating test may fail, especially on using acetic acid, in the presence of only a slight amount of proteid. 2. *Behavior towards Mineral Acids at Ordinary Temperatures.* The proteids are precipitated by the three ordinary mineral acids and by metaphosphoric acid, but not by orthophosphoric acid. If nitric acid be placed in a test tube and the albumin solution be allowed to flow gently thereon, a white, opaque ring of precipitated albumin will form where the two liquids meet (HELLER'S albumin test). 3. *Precipitation by Metallic Salts.* Copper sulphate, neutral and basic lead acetate (in small amounts), mercuric chloride, and other salts precipitate albumin. On this is based the use of albumins as antidotes in poisoning by metallic salts. 4. *Precipitation by Ferro- or Ferricyanide of Potassium in Acetic Acid Solution.* In these tests the relative quantities of reagent, proteid, or acid do not interfere with the delicacy of the test. 5. *Precipitation by Neutral Salts*, such as Na_2SO_4 or NaCl, when added to saturation to the liquid acidified with acetic acid or hydrochloric acid. 6. *Precipitation by Alcohol.* The solution must not be alkaline, but must be either neutral or faintly acid. It must, at the same time, contain a sufficient quantity of neutral salts. 7. *Precipitation by Tannic Acid* in acetic-acid solutions. The absence of neutral salts or the presence of free mineral acids may not cause the precipitate to appear, but after the addition of a sufficient quantity of sodium acetate the precipitate will in both cases

appear. 8. *Precipitation by Phospho-tungstic or Phospho-molybdic Acids* in the presence of free mineral acids. *Potassium-mercuric iodide* and *potassium-bismuth iodide* precipitate albumin solutions acidified with hydrochloric acid. 9. *Precipitation by Picric Acid* in solutions acidified by organic acids. 10. *Precipitation by Trichloracetic Acid*[1] in 2–5% solution.

B. Color Reactions for Proteid Bodies.

1. *Millon's reaction.*[2] A solution of mercury in nitric acid containing some nitrous acid gives a precipitate with proteid solutions which at the ordinary temperature is slowly, but at the boiling-point more quickly, colored red; and the solution may also be colored a feeble or bright red. Solid albuminous bodies, when treated by this reagent, give the same coloration. This reaction, which depends on the presence of the aromatic group in the proteid, is also given by tyrosin and other benzol derivatives with a hydroxyl group in the benzol nucleus.[3] 2. *Xanthoproteic reaction.* With strong nitric acid the albuminous bodies give, on heating to boiling, yellow flakes or a yellow solution. After saturating with ammonia or alkalies the color becomes orange-yellow. 3. *Adamkiewicz' reaction.* If a little proteid is added to a mixture of 1 vol. concentrated sulphuric acid and 2 vols. glacial acetic acid a reddish-violet color is obtained slowly at ordinary temperatures, but more quickly on heating. Gelatine does not give this reaction. 4. *Biuret test.* If a proteid solution be first treated with caustic potash or soda and then a dilute copper sulphate solution be added drop by drop, first a reddish, then a reddish-violet, and lastly a violet-blue color is obtained. 5. Proteids are soluble on heating with *concentrated hydrochloric acid*, producing a violet color, and when they are previously boiled with alcohol and then washed with ether (LIEBERMANN[4]) they give a beautiful blue solution. 6. With *concentrated sulphuric acid* and *sugar* (in small quantities) the albuminous

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[1] F. Obermayer, Wiener med. Jahrbücher, 1888.

[2] The reagent is obtained in the following way: 1 pt. mercury is dissolved in 2 pts. of nitric acid (of sp. gr. 1.42), first when cold and later by warming. After complete solution of the mercury add 1 volume of the solution to 2 vollumes of water. Allow this to stand a few hours and decant the supernatant liquid.

[3] See O. Nasse, Sitzungsber. d. Naturforsch. Gesellsch. zu Halle, 1879.

[4] Centralbl. f. d. med. Wissensch., 1887.

bodies give a beautiful red coloration. These color reactions apply to all albuminous bodies.

Many of these color reactions are obtained as shown by SALKOWSKI[1] by the aromatic splitting products of the proteids. MILLON'S reaction is only obtained by the substances of the phenol group; the XANTHOPROTEIC reaction by the phenol group and skatol or skatolcarbonic acid. ADAMKIEWICZ'S reaction is only given by the indol group, especially skatolcarbonic acid. LIEBERMANN'S reaction is not given by any of the aromatic splitting products.

The delicacy of the same reagent differs for the different albuminous bodies, and on this account it is impossible to give the degree of delicacy for each reaction for all albuminous bodies. Of the precipitation reactions HELLER'S test (if we eliminate the peptones and certain albumoses) is recommended in the first place for its delicacy; though it is not the most delicate reaction, and because it can be performed so easily. Among the precipitation reactions, that with basic lead acetate (when carefully and exactly executed) and the reactions 6, 7, 8, and 9 are the most delicate. The color reactions 1 to 4 show great delicacy in the order in which they are given.

No proteid reaction is in itself characteristic, and, therefore, in testing for proteids one reaction is not sufficient, but a number of precipitation and color reactions must be employed.

For the quantitative estimation of coagulable proteids the determination by boiling with acetic acid can be performed with advantage since, by operating carefully, it gives exact results. Treat the proteid solution with a 1–2% common-salt solution, or if the solution contains large amounts of proteid dilute with the proper quantity of the above salt solution, and then carefully neutralize with acetic acid. Now determine the quantity of acetic acid necessary to completely precipitate the proteids in small measured portions of the neutralized liquid which have previously been heated on the water-bath, so that the filtrate does not respond with HELLER'S test. Now warm a larger weighed or measured quantity of the liquid on the water-bath, and add gradually the required quantity of acetic acid, with constant stirring, and continue the heat for some time. Filter, wash with water, extract with alcohol and then with ether, dry, weigh, incinerate and weigh again. With proper work the filtrate should not give HELLER'S test. This method serves in most cases, and especially so in cases where other bodies are to be quantitatively estimated in the filtrate.

The precipitation by means of alcohol may be used in the quantitative estimation of proteids. The liquid is first carefully neutralized, treated with some NaCl if necessary, and then alcohol added until the solution contains 70–80 vol. per cent anhydrous alcohol. The precipitate is collected on a filter, extracted with alcohol and ether, dried, weighed, incinerated and again weighed. This method is only applicable to liquids which do not contain any other substances, like glycogen, which are insoluble in alcohol.

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[1] Zeitschr. f. physiol. Chem., Bd. 12, S. 215.

In both these methods small quantities of proteids may remain in the filtrates. These traces may be determined as follows: Concentrate the filtrate sufficiently, remove any separated fat by shaking with ether, and then precipitate with tannic acid. Approximately 63% of the tannic acid precipitate, washed with cold water and then dried, may be considered as proteid.

Good results are also obtained by the following method as suggested by DEVOTO.[1] The liquid is treated with 80 gms. crystallized ammonium sulphate for every 100 c.c. of fluid and warmed on the water-bath until the salt dissolves. Then place the vessel in steam for 30–40 minutes to 2 hours, collect the finely divided precipitate on a filter, wash with water until free from sulphates, extract with alcohol and ether, dry and proceed as ordinarily. This method does not give quite exact results with blood or fluids containing blood, but otherwise it seems to be very serviceable.

The quantitative estimation of proteids by means of precipitating with copper sulphate cannot be used in all cases. The same is true for the estimation by means of the polariscope, which does not give sufficiently accurate results.

The removal of proteids from a solution may in most cases be performed by boiling with acetic acid. Small amounts of proteid which remain in the filtrates may be separated by boiling with freshly precipitated lead carbonate or with ferric acetate, as described in Chapter XV (on the urine). If the liquid cannot be boiled, the proteid may be precipitated by the very careful addition of lead acetate, or by the addition of alcohol. If the liquid contains substances which are precipitated by alcohol, such as glycogen, then the proteid may be removed by the alternate addition of potassium-mercuric iodide and hydrochloric acid (see Chapter VIII, on Glycogen Estimation).

Synopsis of the Most Important Properties of the Different Chief Groups of Proteids.

Those proteids which occur formed, in the ordinary sense, in the animal fluids and tissues, and which can be isolated from these without losing their original properties by different chemical means, are called NATIVE PROTEIDS. New modifications, with other properties, may be obtained from these native proteids by the action of heat, various chemical reagents, such as acids, alkalies, alcohol, and others, as also by proteolytic enzymes. These new proteids are called MODIFIED[1] PROTEIDS, in contradistinction to the native proteids. The albumins, globulins, and nucleoalbumins, as given in the scheme on page 18, belong to the native proteids, while the acid and alkali albuminates, albumoses, peptones, and the coagulated proteids belong to the modified proteids.

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[1] Zeitschr. f. physiol. Chem., Bd. 15, S. 465.

The native proteids may be precipitated by sufficient amounts of neutral salts without changing their properties, although the various proteids act differently with different neutral salts. Some are precipitated by NaCl, others only by $MgSO_4$, and still others by only $(NH_4)_2SO_4$, which is the precipitant for nearly all proteids. These various properties, as also the different solubility in water and dilute salt solution, are used at the present time to differentiate between the various proteids and groups, although it must be stated that these differences are only relative and are often uncertain.

Albumins. These bodies are soluble in water and are not precipitated by the addition of a little acid or alkali. They are precipitated by the addition of large quantities of mineral acids or metallic salts. Their solution in water coagulates on boiling in the presence of neutral salts, but a weak saline solution does not. If NaCl or $MgSO_4$ is added to saturation to a neutral solution in water at the normal temperature or at + 30° C. no precipitate is formed; but if acetic acid is added to this saturated solution the albumin readily separates. When ammonium sulphate is added in substance to saturation to an albumin solution a complete precipitation occurs at ordinary temperature. Of all the albuminous bodies the albumins are the richest in sulphur, containing from 1.6% to 2.2%.

Globulins. These albuminous bodies are insoluble in water, but dissolve in dilute neutral salt solutions. The globulins are precipitated unchanged from these solutions by sufficient dilution with water, and on heating they coagulate. The globulins dissolve in water on the addition of very little acid or alkali, and on neutralizing the solvent they precipitate again.

The solution in a minimum amount of alkali is precipitated by carbon dioxide, but the precipitate may be redissolved by an excess of the precipitant. The neutral solutions of the globulins containing salts are partly or completely precipitated on saturation with

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[1] The word *denaturierung* as used by Neumeister and the author is translated by the word *modified*, as it best expresses the meaning. The word *derived* might also be used.

NaCl or $MgSO_4$ in substance at normal temperatures. The globulins are completely precipitated by saturating with ammonium sulphate. The globulins contain an average amount of sulphur, not below 1%.

A sharp line between the globulins on one side and the artificial albuminates on the other can hardly be drawn. The albuminates are, indeed, as a rule insoluble in dilute common-salt solutions; but an albuminate may be prepared by the action of strong alkali which is soluble in common-salt solutions immediately after precipitation. We also have globulins which are insoluble in NaCl after having been in contact with water for some time.

Nucleoalbumins. These bodies are found widely diffused in both the animal and vegetable kingdoms. They form one of the chief constituents of protoplasm, while the albumins and in part also the globulins are special constituents of the animal juices. The nucleoalbumins are found in organs abounding in cells, but they also occur in secretions and sometimes in other fluids in apparent solution as destroyed and altered protoplasm. The nucleoalbumins behave like rather strong acids; they are nearly insoluble in water, but dissolve easily with the aid of a little alkali. Such a solution, neutral or, indeed, a faintly acid one, does not coagulate on boiling. The nucleoalbumins resemble the globulins and the albuminates in solubility and precipitation properties, but differ from them in being hardly soluble in neutral salts. The most important difference between the nucleoalbumins, the globulins, and the albuminates is that the nucleoalbumins contain phosphorus, and by the action of pepsin hydrochloric acid on nucleoalbumins a phosphorized product, *paranuclein* or *pseudonuclein*, is split off which, according to LIEBERMANN,[1] is a combination of albumin with metaphosphoric acid. The nucleoalbumins seem habitually to contain less sulphur than the bodies of the preceding groups. Some iron is found as a constant constituent.

The nucleoalbumins are often confounded with nucleoproteids and also with phosphorized glycoproteids. From the first class they differ by not yielding any xanthin bodies when boiled with acids, and from the second group by not yielding any reducing substance on the same treatment.

Lecithalbumins. On the preparation of certain protein substances products are often obtained containing lecithin, and this lecithin can only be removed with difficulty or incompletely by a mixture of alcohol and ether. Ovovitellin is such a protein body containing considerable lecithin, and HOPPE-SEYLER[1] considers it a combination of albumin and lecithin. LIEBERMANN[2] has obtained proteids containing lecithin as an insoluble residue on the peptic digestion of mucous membranes of the stomach, liver, kidneys, lungs, and spleen. He considers them as combinations of proteid and lecithin and calls them *lecithalbumins*. They differ from the nucleo-albumins in that no metaphosphoric acid is split off and from the nucleo-proteids for the same reason, and also in that they do not yield xanthin bases. Further investigations are necessary on this subject.

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[1] Ber. d. deutsch. chem. Gesellsch., Bd. 21.

Alkali and Acid Albuminates. By the action of alkalies all native albuminous bodies are converted, with the elimination of nitrogen or by the action of stronger alkali with the emission of sulphur, into a new modification, called alkali albuminate, whose specific rotation is increased at the same time. If caustic alkali in substance or in strong solution be allowed to act on a concentrated proteid solution, such as blood-serum or egg-albumin, the alkali albuminate may be obtained as a solid jelly which dissolves in water on heating, and which is called "LIEBERKÜHN's solid alkali albuminate." By the action of dilute caustic alkali solutions on dilute proteid solutions we have alkali albuminates formed slowly at the ordinary temperature, but more rapidly on heating. These solutions may be modified by the source of the proteid acted upon, and also by the extent of the action of the alkali, but still they have certain reactions in common.

If proteid is dissolved in an excess of concentrated hydrochloric acid, or if we digest a proteid solution acidified with 1–2 p. m. hydrochloric acid in the warmth, or digest the proteid alone with pepsin hydrochloric acid, we obtain new modifications of proteid which indeed may show somewhat varying properties, but have certain reactions in common. These modifications, which may be obtained in a solid gelatinous condition on sufficient concentration, are called acid albuminates or acid albumins, sometimes also syntonin, though we prefer to call that acid albuminate syntonin which is obtained by extracting muscles with hydrochloric acid of 1 p. m.

The alkali and acid albuminates have the following reactions in common: They are nearly insoluble in water and dilute common-salt solutions (see page 31), but they dissolve readily in water on the addition of a very small quantity of acid or alkali. Such a solution or one nearly neutral does not coagulate on boiling if neutral salts are not present in sufficient quantity, but is precipitated at the normal temperature on neutralizing the solvent by an alkali or an acid. A solution of an alkali or acid albuminate in acid is easily precipitated on saturating with NaCl, but a solution in alkali is precipitated with difficulty or not at all, according to the amount of alkali it contains. The nearly neutral solutions are precipitated by mineral acids in excess, also by many metallic salts.

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[1] Hoppe-Seyler, Med. chem. Untersuch., 1868; also Zeitschr. f. physiol. Chem., Bd. 13, S. 479.

[2] Pflüger's Archiv, Bdd. 50 and 54.

Notwithstanding this agreement in the reactions, the acid and alkali albuminates are essentially different, and by dissolving an alkali albuminate in some acid no acid albuminate solution is obtained, nor is an alkali albuminate formed on dissolving an acid albuminate in water by the aid of a little alkali. The alkali albuminates are relatively strong acids. They may be dissolved in water with the addition of $CaCO_3$, with the elimination of CO_2, which does not occur with typical acid albuminates, and they show in opposition to the acid albuminates also other variations which stand in connection with their strongly marked acid nature. Dilute solutions of alkalies act more energetically on proteids than do acids of corresponding concentration. In the first case a part of the nitrogen, and often also the sulphur, is split off, and from this property we may obtain an alkali albuminate by the action of an alkali upon an acid albuminate; but we cannot obtain an acid albuminate by the reverse reaction. (K. MÖRNER.[1])

The preparation of the albuminates has been given above. By the action of alkalies or acids upon an proteid solution the corresponding albuminate may be precipitated by neutralizing with acid or alkali. The washed precipitate is dissolved in water by the aid of a little alkali or acid, and again precipitated by neutralizing the solvent. If this precipitate which has been washed in water is treated with alcohol and ether, the albuminate will be obtained in a pure form.

Albumoses and Peptones. Peptones are designated as the final products of the decomposition of albuminous bodies by means of proteolytic enzymes, in so far as these final products are still true albuminous bodies, while we designate as albumoses, proteoses, or propeptones the intermediate products produced in the peptonization of proteids in so far as they are substances not similar to albuminates.

Albumoses and peptones may also be produced by the hydrolytic decomposition of the proteids with acids or alkalies, also by the

[1] Pflüger's Archiv, Bd. 17.

putrefaction of the same. They may also be formed in very small quantities as by-products in the investigations of animal fluids and tissues, and the question to what extent these exist preformed under physiological conditions requires very careful investigation.

Between the peptones which represent the last splitting products and those albumoses which stand closest to the original proteids we have undoubtedly a series of intermediate products. Under such circumstances it is a difficult problem to try to draw a sharp line between the peptone and the albumose group, and it is just as difficult to define our conception of peptones and albumoses in an exact and satisfactory manner.

The *albumoses* have been considered as those albuminous bodies whose neutral or faintly acid solutions do not coagulate on boiling and which, to distinguish them from peptones, were characterized chiefly by the following properties. The watery solutions are precipitated at the ordinary temperature by nitric acid as well as by acetic acid and potassium ferrocyanide, and this precipitate has the peculiarity of disappearing on heating and reappearing on cooling. If a solution of albumoses is saturated with NaCl in substance, the albumoses are partly precipitated in neutral solutions, but on the addition of acid saturated with the salt they completely precipitate. This precipitate, which dissolves on warming, is a combination of albumose with the acid.

We formerly designated as *peptone* those proteid bodies which are readily soluble in water and which do not coagulate by heat, whose solutions are precipitated neither by nitric acid, nor by acetic acid and potassium ferrocyanide, nor by neutral salts and acid.

The reactions and properties which the albumoses and peptones had in common were formerly considered as the following: They give all the color reactions of the proteids, but with the biuret test they give a more beautiful red color than the ordinary proteids. They are precipitated by ammoniacal lead acetate, by mercuric chloride, tannic, phospho-tungstic, phospho-molybdic acids, potassium-mercuric iodide and hydrochloric acid, and lastly by picric acid. They are precipitated but not coagulated by alcohol, namely, the precipitate obtained is soluble in water even after being in contact with alcohol for a long time. The albumoses and peptones also have a greater diffusive power than native albuminous bodies, and the diffusive power is greater the nearer the questionable substance stands to the final product, the now so-called pure peptone.

These old views have undergone an essential change in the last few years. After HEYNSIUS'[1] observation that ammonium sulphate was a general precipitant for proteids, also peptone in the old sense, KÜHNE[2] and his pupils proposed this salt as a means of separating albumoses and peptones. Those products of digestion which separate on saturating their solution with ammonium sulphate are considered by KÜHNE and indeed by most of the modern investigators as albumoses, while those which remain in solution are called peptones or pure peptone. This pure peptone is formed in relatively large amounts in pancreatic digestion, while in pepsin digestion it is only formed in small quantities or after prolonged digestion.

According to SCHÜTZENBERGER[3] and KÜHNE[4] the proteids yield two chief groups of new albuminous bodies when decomposed by dilute mineral acids or with proteolytic enzymes; of these the *anti group* shows a greater resistance to further action of the acid and enzyme than the other, namely, the *hemi group*. Corresponding to these views KÜHNE divides the albumoses into two chief groups, the *antialbumoses* and *hemialbumoses*, and the peptones into two chief groups, the *antipeptones* and the *hemipeptones*. In pepsin digestion we obtain, besides different albumoses, a mixture of anti- and hemipeptone, which mixture KÜHNE called *amphopeptone*. In the digestion with trypsin (the proteolytic enzyme of the pancreas) the hemipeptone is further split into leucin, tyrosin, and other substances, while the antipeptone remains unchanged. By the sufficiently energetic action of trypsin only one peptone is at last obtained, the so-called antipeptone.

KÜHNE and his pupils, who have conducted these complete investigations on the albumoses and peptones, classify the various albumoses according to their different solubilities and precipitation powers. In the pepsin digestion of fibrin[5] they obtained the following albumoses: (*a*) *Heteroalbumose*, insoluble in water but soluble in dilute salt solution; (*b*) *Protalbumose*, soluble in salt solution and water. These two albumoses are precipitated by NaCl in neutral solutions, but not completely. Heteroalbumose may be converted into a modification, called (*c*) *Dysalbumose*, which is insoluble in dilute salt solutions by being in contact with water for a long time or by drying. (*d*) *Deuteroalbumose* is an albumose which is soluble in water and dilute salt solution and which is incompletely precipitated from acid solution by saturating with NaCl and not precipitated from neutral solutions. This precipitate is a combination of the albumose with acid (HERTH[1]).

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[1] Pflüger's Archiv, Bd. 34.

[2] See Kühne, Verhandl. d. naturhistor. Vereins zu Heidelberg (N. F.), 3; J. Wenz, Zeitschr. f. Biologie, Bd. 22; Kühne and Chittenden, Zeitschr. f. Biologie, Bd. 22; R. Neumeister, *ibid.*, Bd. 23; Kühne, *ibid.*, Bd. 29.

[3] Bull. de la soc. chimique de Paris, 23.

[4] See Kühne, Verhandl. d. naturhistor. Vereins zu Heidelberg (N. F.), Bd. 1, and Kühne and Chittenden, Zeitschr. f. Biologie, Bd. 19.

[5] See Kühne and Chittenden, Zeitschr. f. Biologie, Bd. 20.

HERTH[1] claims that the relative proportion of acid or alkali, salt, water, or albumose in a solution essentially changes the solubility and precipitation power of the same. He also claims that the occurrence of several different kinds of albumoses cannot be demonstrated, because with one and the same albumose, the above conditions being changed, its solubilities and precipitating powers are changed. HAMBURGER[2] found the same to be true from his investigations.

The albumoses obtained from different proteid bodies do not seem to be identical, but differ in their behavior to precipitants. Special names have been given to these various albumoses according to the mother proteid, namely, *globuloses*,[3] *vitilloses*,[4] *caseoses*,[5] *myosinoses*,[6] etc. These various albumoses are further distinguished, as *proto-*, *hetero-*, and *deutero*-caseoses for example. All the albumoses formed in the digestion of animal and vegetable proteid are embraced in the common name *proteoses* by CHITTENDEN.[7]

NEUMEISTER[8] designates as *atmidalbumose* that body which is obtained by the action of superheated steam on fibrin. At the same time he also obtained a substance called *atmidalbumin* which stands between the albuminates and the albumoses. CHITTENDEN and FRANK[9] have obtained as products of the action of superheated steam on ovalbumin, besides a little peptone, leucin, and tyrosin, two substances, similar to albumoses, which correspond to the two atmid substances of NEUMEISTER, but differ from them by containing a higher percentage of carbon.

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[1] Monatshefte f. Chem., Bd. 5.

[2] See Maly's Jahresber., Bd. 16, S. 20.

[3] Kühne and Chittenden, Zeitschr. f. Biologie, Bd. 22.

[4] Neumeister, *ibid.*, Bd. 32; Chittenden and Hartwell, Journ. of Physiol., Vol. 11.

[5] Chittenden and Painter, Studies from the Laboratory, etc., Yale University, Vol. 2, New Haven, 1891; Chittenden, *ibid.*, Vol. 3; Sebelein, Chem. Centralblatt, 1890.

[6] Kühne and Chittenden, Zeitschr. f. Biologie, Bd. 25; Chittenden and Goodwin, Journ. of Physiol., Vol. 12.

[7] Chittenden and Hartwell, Journ. of Physiol., Vol. 12.

[8] Zeitschr. f. Biologie, Bd. 26.

[9] Journal of Physiol., Vol. 15.

Of the soluble albumoses NEUMEISTER[1] designates protoalbumose and heteroalbumose as *primary albumoses*, while the deuteroalbumoses, which are closely allied to the peptones, he calls *secondary albumoses*. As essential difference between the primary and secondary albumoses he suggests the following:[2] The primary albumoses are precipitated by nitric acid in salt-free solutions, while the secondary albumoses are only precipitated in salt solutions, while certain deuteroalbumoses, such as deuterovitillose and deuteromyosinose, are only precipitated by nitric acid in solutions saturated with NaCl. The primary albumoses are precipitated from neutral solutions by copper sulphate solution (2 : 100), also by NaCl in substance, while the secondary albumoses are not. The primary albumoses are completely precipitated from their solution saturated with NaCl by the addition of acetic acid saturated with salt, while the secondary albumoses are only partly precipitated. The primary albumoses are readily precipitated by acetic acid and potassium ferrocyanide, while the secondary are only incompletely precipitated after some time. The deuteroalbumoses are derived from the primary albumoses and therefore have a smaller molecular weight. Contrary to this view KÜHNE[3] has found that deuterofibrinoses diffuse less readily than the protofibrinose, and also, according to SABANEJEW,[4] the deuteroalbumose has a higher molecular weight (3200) than the protoalbumose (2467–2643).

PAAL[5] has prepared combinations of peptone, from ovalbumin, with hydrochloric acid in a manner similar to that with gelatine. The elementary composition of the different preparations showed considerable variation, as did also the molecular weight. The acid combining power of the hydration products produced in peptonization increased with the progress of the hydrolytic cleavage. SCHRÖTTER[6] has prepared from WITTE's albumose mixture a crystalline albumose, separating from methyl alcohol on cooling, whose hydrochloride contained on an average 10.8% HCl and whose molecular weight was 587–714 as determined by RAOULT's method. As the electrical conductivity of hydrochloric acid diminishes in proportion as the acid is neutralized by alkali, so SJÖQVIST[1] found a similar behavior when hydrochloric acid was neutralized with proteids. Starting from these circumstances SJOQVIST has studied the combinations of proteids with HCl, HNO_3, H_2SO_4, and H_3PO_4, and has tried to determine the chemical equivalent of proteids. He found this to be about 800 for ovalbumin, about 600 for albumose, and about 250 for peptone.

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[1] Zeitschr. f. Biologie, Bd. 24.

[2] *Ibid.*, Bd. 26.

[3] *Ibid.*, Bd. 29.

[4] Ber. d. deutsch. chem. Gesellsch., Bd. 26, Ref. p. 385.

[5] *Ibid.*, Bd. 27.

[6] Monatshefte f. Chem., Bd. 14.

The true peptones are exceedingly hygroscopic, and when perfectly dry sizzle like phosphoric anhydride when treated with water. They are exceedingly soluble in water, diffuse more readily than the albumoses, and are not precipitated by ammonium sulphate. Pure true peptones are not precipitated either by picric acid or by potassium-mercuric iodide and acid. They are incompletely precipitated by phospho-tungstic or phospho-molybdic acids. The peptones are precipitated by tannic acid, but this may be redissolved in an excess of the precipitant (SEBELIEN[2]). According to SABANEJEW the molecular weight of the peptones is below 400.

As the so-called true peptones hitherto have not been prepared perfectly pure, and therefore the characteristic properties are still not known, we consider the behavior to ammonium sulphate as the absolute difference between albumoses and peptones. It is still doubtful whether the behavior of a single salt, the ammonium sulphate, yields sufficient basis for the characterization of two groups of albuminous bodies, the albumoses and peptones; and this question is warranted since, according to NEUMEISTER, we have a deuteroalbumose (formed from the protalbumose in peptic digestion) which is not completely precipitated by ammonium sulphate. It seems that the transformation of proteids into peptones takes place through a number of intermediate steps similar to the transformation of starch into dextrose through a series of dextrins. A complete separation of these several intermediate products as well as their purification is such an extremely difficult task that it is nearly impossible at present to say how far such a differentiation is warranted or feasible.

What relationship do the albumoses and peptones bear to the proteid from which they are formed? HERTH[3] has found that fibrin albumose and fibrin have approximately the same constitution. KÜHNE and CHITTENDEN, as also CHITTENDEN and his pupils,[1] have analyzed the different albumoses from fibrin, globulin, ovalbumin, myosin, and casein, and found in certain albumoses an increase and in others a decrease in the amount of carbon, nitrogen, and sulphur as compared with the mother-proteid. From the results of their analyses it has been found that, with the probable exception of the albumoses standing closest to the peptone, the difference in the constitution of the original proteids and the corresponding albumoses is sometimes in one direction and sometimes in another, and is at all events unessential.

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[1] Skand. Arch. f. Physiol., Bd. 5.

[2] Chem. Centralbl., 1890.

[3] Zeitschr. f physiol. Chem., Bd. 1, and Monatshefte f. Chem., Bd. 5.

According to the analyses of peptones (in the old sense) made by MALY,[2] HERTH,[3] and HENNINGER,[4] they seem to have the same constitution as the proteid. According to the analyses by KÜHNE and CHITTENDEN[5] of "true" fibrin peptone, part amphopeptone and part antipeptone prepared by pancreas infusion, this peptone was found to contain about the same amount of hydrogen and the same or a greater amount of nitrogen, but considerably less carbon than the albumoses. In his investigations on casein CHITTENDEN found, on the other hand, that in antipeptone the amount of carbon was higher than in certain caseoses. As the preparation of true peptones in a pure condition is accompanied with great difficulty, and as the peptones (in the modern sense) analyzed have not always behaved as true peptones towards the peptone reagents as described by NEUMEISTER, it is most difficult to draw any positive conclusion from these analyses. It seems, nevertheless, that generally the so-called true peptones are perhaps somewhat poorer in carbon than the corresponding proteids.

The elementary analyses made up to the present time have not given us a positive answer in regard to the relationship existing between the proteids on one side and the albumoses and peptones on the other. The view that the peptone formation is a hydrolytic splitting is accepted by HOPPE-SEYLER,[6] KÜHNE, HENNINGER,[4] and indeed by recent investigators. In support of this view we have the observations of HENNINGER[4] and HOFMEISTER,[7] according to which peptones are converted into a proteid similar to albuminates by the action of acetic-acid anhydride, or by heating so that water is expelled. According to other investigators, as MALY,[1] HERTH,[2] LOEW,[1] and others, the formation of peptone is a depolymerization of the proteids. A third view is that proteids and peptones are isomeric bodies; while a fourth view (GRIESSMAYER[2]) claims that the proteids consist of micell groups which on peptonization are first converted into micelli and then further into molecules. Though an ordinary albumin solution contains micelli or micell bonds, so also a peptone solution contains a proteid molecule.

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[1] See references cited page 36 by Kühne and Chittenden.

[2] Pflüger's Archiv, Bdd. 9 and 20.

[3] Zeitschr. f. physiol. Chem., Bd. 1, and Monatshefte f. Chem., Bd. 5.

[4] Comptes rendus, Tome 86.

[5] See references page 36 by Kühne, Chittenden.

[6] Hoppe-Seyler, Physiol. Chem. Berlin, 1881

[7] Zeitschr. f. physiol. Chem., Bd. 2.

The preparation of different albumoses in a perfectly pure form is very troublesome and accompanied with a great many difficulties. For this reason there will be given here only the general methods by which the different albumose precipitates are obtained. If we proceed from a solution of fibrin in pepsin hydrochloric acid, we first remove the syntonin or some coagulable proteid present by first neutralizing and then coagulating by heat. The neutral filtrate is saturated with NaCl, which precipitates a mixture of primary albumoses. This precipitate is washed with a saturated NaCl solution, pressed and dissolved in dilute salt solution. An insoluble residue remains, which is called dysalbumose. The solution of the primary albumoses is repeatedly and completely dialyzed. Heteroalbumose separates out, while the protalbumose remains in solution and may be precipitated by alcohol. The above filtrate, which has had the primary albumoses removed and saturated with NaCl, is treated with acetic acid, which has previously been saturated with NaCl, until no further precipitate occurs. This precipitate, which consists of a mixture of primary and secondary albumoses, is filtered off, the filtrate freed from salt by dialysis, and the deuteroalbumose precipitated by ammonium sulphate. The various albumoses may also be precipitated from the original solution by ammonium sulphate, dissolved in water and freed from ammonium sulphate by means of dialysis, and then separated as above described.

In the preparation of true peptone we make use of a prolonged pepsin digestion, but much quicker results are obtained by the use of trypsin digestion. The albumoses must be entirely removed, which is done by alternately precipitating in acid, neutral and alkaline solution, with ammonium sulphate. According to KÜHNE[3] we proceed in the following way: The sufficiently dilute and neutral solution (free from albuminates and coagulable proteids) is first precipitated, while boiling hot, with ammonium sulphate. On cooling the precipitated albumoses and crystallized salt are removed by filtration and the filtrate heated to boiling, made strongly alkaline with ammonia and ammonium carbonate, again saturated with ammonium sulphate at the boiling temperature. Remove precipitate by filtration when cold, heat the filtrate again until all odor of ammonia is expelled, saturate with ammonium sulphate while hot, and acidify with acetic acid and filter on cooling.

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[1] Pflüger's Archiv, Bd. 31.

[2] See Maly's Jahresber., Bd. 14, S. 26.

[3] Zeitschr. f. Biologie, Bd. 29.

The filtrate is freed from a great part of the salt by strongly concentrating the liquid, allowing it to cool, and removing the salt by filtration. Another large portion of the salt may be removed from this filtrate by the careful fractional precipitation with alcohol, which yields an alcoholic solution rich in peptone with only a small quantity of ammonium salt. This solution is boiled to remove the alcohol, and then boiled with barium carbonate to remove the ammonium sulphate. The filtrate is freed from excess of barium by the careful addition of dilute sulphuric acid. This filtrate, which must not contain an excess of sulphuric acid, is now concentrated and the peptone precipitated therefrom by alcohol.

For the detection of albumoses and peptones in animal fluids or in watery extracts of organs and tissues we proceed as follows, according to DEVOTO:[1] The coagulable proteids are removed by heating with as pure ammonium sulphate as possible, as above described (page 29). True peptones (besides deuteroalbumose not precipitated) may be detected in the cold filtrate by means of the biuret test. The albumoses are contained in the mixture of precipitate and salt crystals collected on the filter. The albumoses are dissolved from this mixture by washing with water, and may be detected in the wash-water by means of the biuret test. The question as to the possibility of the formation of traces of albumoses from other proteids during this treatment under certain circumstances has not been closely investigated as yet.

If a solution saturated with ammonium sulphate is to be tested by the biuret test, it must first be treated with a slight excess of concentrated caustic-soda solution, keeping the solution cold, and after the sodium sulphate has settled the liquid is treated with a 2% solution of copper sulphate, drop by drop.

The biuret test (colorimetric) and the polariscopic method have been used in the quantitative estimation of albumoses and peptones. These methods do not yield exact results.

Coagulated Proteids.—Proteids may be converted into the coagulated condition by different means: by heating (see page 25), by the action of alcohol, especially in the presence of neutral salts, and in certain cases, as in the conversion of fibrinogen into fibrin (Chapter VI), by the action of an enzyme. RAMSDEN[2] has shown that a proteid solution may also be coagulated by continuous shaking, and indeed in a few cases (ovalbumin) it may be completely coagulated. This coagulation is, however, not identical with heat coagulation. The nature of the processes which take place during coagulation is unknown. The coagulated albuminous bodies are insoluble in water, in neutral salt solutions, and in dilute acids or alkalies, at normal temperature. They are dissolved and converted into albuminates by the action of less dilute acids or alkalies, especially on heating.

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[1] Zeitschr. f. physiol. Chem., Bd. 15.

[2] Du Bois-Reymond's Arch., 1894.

Coagulated proteids appear also to occur in animal tissues. We find, at least in many organs such as the liver and other glands, proteids which are not soluble in water, dilute salt solutions, or very dilute alkalies, and only dissolve after being modified by strong alkalies.

Appendix.

Vegetable Proteids. Vegetable proteids seem to have the same essential properties as the animal proteids, and the three chief groups of native proteids occur in the plants as well as the animal organism. We recognize the following as vegetable proteids: *albumins*, *globulins* (phytovitellin, vegetable myosin, paraglobulin), and *nucleoalbumins* (pea legumin). Besides these a special group of coagulated proteids, so-called gluten proteins, occur, which are partly soluble in alcohol. It seems that too much importance is given to the solubilities of the vegetable proteids, and more exhaustive investigations seem to be necessary.[1]

Poisonous Proteids. Attention was called in the first chapter to the fact that high plants and animals, as well as microbes, can produce proteids having specific, sometimes intense, poisonous action.

We know very little positively in regard to the nature of these proteids. Those which have been isolated belong to certain of the proteid groups—some are albumins, others globulins or compound proteids, and the majority seem to be albumoses—still little is known in regard to their chemical nature. From a chemical standpoint we do not differentiate between a poisonous and a harmless proteid; for example, between a poisonous and a non-poisonous globulin. The fundamental question whether those that have been isolated as poisonous proteids are really poisonous or not, or whether they consist of a harmless proteid contaminated with a poisonous substance, cannot be considered as settled.

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[1] See Kjeldahl: Undersögelser over de optiske Forhold hos nogle Planteæggehvidestoffer. Forhandlingerne ved de skandinaviske Naturforskeres 14. Möde. Kiöbenhavn, 1892.

One thing is certain, and that is that one and the same toxalbumin can show essentially different chemical properties under different circumstances, although it shows the same specific action. Tuberculin is an example of this kind. This, according to most investigators, is an albumose; but contrary to this HELMAN[1] has isolated a tuberculin which does not act like an albumose and on the whole only gives faint proteid reactions. The elementary composition of one and the same toxalbumin, prepared in different ways, also shows considerable variations.[2]

Under such circumstances, nothing definite can be stated in regard to the properties of the different toxalbumins. The study of the nature of poisonous proteids seems to be in the same state as the study of the enzymes, and we cannot deny that in many cases an unmistakable similarity of action is observed between toxalbumins and enzymes.

II. Compound Proteids.

With this name we designate a class of bodies which are more complex than the simple proteids and which yield as nearest splitting products simple proteids on one side and non-proteid bodies, such as coloring matters, carbohydrates, xanthin bases, etc., on the other.[3]

The compound proteids known at the present time are divided into three chief groups. These groups are the *hæmoglobins*, the *glycoproteids*, and the *nucleoproteids*. The hæmoglobins will be treated of in a following chapter (Chapter VI, on the blood).

Glycoproteids are those compound proteids which on decomposition yield a proteid on one side and a carbohydrate or derivatives of the same on the other. Some glycoproteids are free from phosphorus (mucins, mucinoids, and hyalogens), and some contain phosphorus (phosphoglycoproteids).

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[1] Archives de sciences biologiques de St. Petersbourg. Tome 1, 1892.

[2] See S. Dzierzgowski and L. de Rekowski: Recherches sur la transformation des milieux nutritifs par les bacilles de la diphthérie, etc. Archives de sciences biologiques de St. Petersbourg. Tome 1, 1892.

[3] Hoppe-Seyler has given the name *proteïde* to these compound proteids, but as this term is misleading in English we do not use it in English classifications in this sense.

Mucin Substances. We designate as mucins colloid substances whose solutions are mucilaginous and thready, and which when treated with acetic acid give a precipitate insoluble in an excess of acid, and on boiling with dilute mineral acids yield a substance capable of reducing copper oxyhydrate. This last-mentioned fact, which was first observed by EICHWALD,[1] differentiates mucins from other bodies which have long been mistaken for it and which have similar physical properties. On the other hand, bodies whose physical properties differ from it, but which give a reducible substance on boiling with dilute mineral acids, have also been designated as mucins.

The different bodies characterized as mucin substances correspond, first, either to *true mucins*, or, second, to *mucoids* or *mucinoids*.

All mucin substances contain *carbon*, *hydrogen*, *nitrogen*, *sulphur*, and *oxygen*. Compared with albuminous bodies they contain less nitrogen and, as a rule, considerably less carbon. As immediate decomposition products they yield albuminous bodies on one side and carbohydrates or acids allied thereto on the other. On boiling with dilute mineral acids they all give a reducing substance.

The *true mucins* are characterized by their natural solution, or one prepared by the aid of a trace of alkali, being mucilaginous, thread-like, and giving a precipitate with acetic acid which is insoluble in excess of acid. The *mucoids* do not show these physical properties and have other solubilities and precipitation properties. As we have intermediate steps between different albuminous bodies, so also we have such between true mucins and mucoids, and a sharp line between these two groups cannot be drawn.

True mucins are secreted by the larger mucous glands, by certain mucous membranes, also by the skin of snails and other animals. True mucin also occurs in the connective tissue and navel-cord. Sometimes, as in snails and in the membrane of the frog-egg (GIACOSA[2]), a mother-substance of mucin, a mucinogen, has been found which may be converted into mucin by alkalies.

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[1] Annal. d. Chem. u. Pharm., Bd. 184.

[2] Zeitschr. f. physiol. Chem., Bd. 7; also Hammarsten, Pflüger's Archiv, Bd. 36.

Mucoid substances are found in cartilage, certain cysts, in the cornea, the crystalline lens, white of egg, and in certain ascitic fluids. As the mucin question has been very little studied, it is at the present time impossible to give any positive statements in regard to the occurrence of mucins and mucoids, especially as without doubt in many cases non-mucinous substances have been described as mucins. So much is sure, that mucins or nearly related bodies occur widely diffused in the organism in certain tissues. From their decomposition products we derive a great deal of knowledge in regard to the formation and splitting of carbohydrates or kindred bodies (glycuronic acid) from other complex groups.

True Mucins. Thus far we have been able to obtain only a few mucins in a pure and unchanged condition due to the reagents used. The elementary analyses of these mucins have given the following results:

	C	H	N	S	O	
Mucin from snail...........	50.32	6.84	13.65	1.75	27.44	(Hammarsten)[1]
Mucin from tendon.........	48.30	6.44	11.75	0.81	32.70	(Loebisch)[2]
Mucin from submaxillary...	48.84	6.80	12.32	0.84	31.20	(Hammarsten)[3]

The mucin of the snail-skin, which stands closest to keratin, contains more sulphur than the other mucins. The sulphur is moreover, at least in certain mucins, part in loose and part in strong chemical union.

By the action of superheated steam on mucin a carbohydrate, animal gum (Landwehr[4]), is split off. This is not essentially true for all mucins, as the mucin from the submaxillary gland yields a gummy substance containing nitrogen.[5]

On boiling mucin with dilute mineral acids, acid albuminate and bodies similar to albumose or peptone are obtained, besides a reducing substance which has not been closely studied. By the action of stronger acids we obtain among other bodies leucin, tyrosin, and levulinic acid (Landwehr). Certain mucins, as the submaxillary mucin, are easily changed by very dilute alkalies, as lime-water, while others, such as tendon-mucin, are not affected (Loebisch[6]). If a strong caustic-alkali solution, as a 5% KOH solution, is allowed to act on submaxillary mucin, we obtain alkali albuminate, a body similar to albumose and peptone, and one or more substances of an acid reaction and with strong reducing powers.

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[1] Pflüger's Archiv, Bd. 36.

[2] Zeitschr. f. physiol. Chem., Bd. 10.

[3] Zeitschr. f. physiol. Chem., Bd. 12.

[4] Zeitschr. f. physiol. Chem., Bdd. 8 and 9; also Pflüger's Archiv, Bdd. 39 and 40.

[5] Not officially published by the author.

[6] Zeitschr. f. physiol. Chem., Bd. 10.

In one or the other respect the different mucins act somewhat differently. For example, the snail and tendon mucins are insoluble in dilute hydrochloric acid of 1–2 p. m., while the mucin of the submaxillary gland and the naval-cord are soluble. Tendon-mucin becomes flaky with acetic acid, while the other mucins are precipitated in more or less fibrous, tough masses. Still all the mucins have certain reactions in common.

In the dry state mucin forms a white or yellowish-gray powder. When moist it forms, on the contrary, flakes or yellowish-white tough lumps or masses. The mucins are acid in reaction. They give the color reactions of the albuminous bodies. They are not soluble in water, but may give a neutral solution with water and the smallest quantity of alkali. Such a solution does not coagulate on boiling, while acetic acid gives at the normal temperature a precipitate which is insoluble in an excess of the precipitant. If 5–10% NaCl be added to a mucin solution, this can now be carefully acidified with acetic acid without giving a precipitate. Such acidified solutions are copiously precipitated by tannic acid; with potassium ferrocyanide they give no precipitate, but on sufficient concentration they become thick or viscous. A neutral solution of mucin-alkali is precipitated by alcohol in the presence of neutral salts; it is also precipitated by several metallic salts. If mucin is heated on the water-bath with dilute hydrochloric acid of about 2%, the liquid gradually becomes a yellowish or dark brown and reduces copper oxyhydrate from alkaline solutions.

The mucin most readily obtained in large quantities is the submaxillary mucin, which may be prepared in the following way: The filtered watery extract of the gland, free from form-elements and as colorless as possible, is treated with 25% hydrochloric acid, so that the liquid contains 1.5 p. m. HCl. On the addition of the acid the mucin is immediately precipitated, but dissolves on stirring. If this acid liquid is immediately diluted with 2–3 vols. of water, the mucin separates and may be purified by redissolving in 1–5 p. m. acid, and diluting with water and washing therewith. The mucin of the naval-cord may be prepared in the same way.[1] The tendon-mucin is prepared from tendons which have first been freed from proteid by common-salt solution and water. They are extracted with lime-water, the filtrate is precipitated with acetic acid, and the precipitate purified by redissolving in dilute alkali or lime-water, precipitating with acid, and washing with water (ROLLETT,[1] LOEBISCH). Lastly, the mucins are treated with alcohol and ether.

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[1] The author has not been able to obtain this pure, so the analysis has not been given in the previous table of the mucins.

Mucoids or **Mucinoids.** To this group belong *pseudomucin*, which occurs in ovarial liquids, *colloid*, which is probably related thereto, and *chondromucoid*, which occurs in cartilage, and others. These bodies will be treated of later in their respective chapters.

Hyalogens. Under this name KRUKENBERG[2] has designated a number of differing bodies, which are characterized by the following: By the action of alkalies they change, with the splitting off of sulphur and some nitrogen, into soluble nitrogenized products called by him *hyalines* and which yield a pure carbohydrate by further decomposition. We find that very heterogeneous substances are included in these groups. Certain of these hyalogens seem undoubtedly to be glycoproteids. *Neossin*[3] of the Chinese edible swallow's-nest, *membranin*[4] of DESCEMET'S membrane and of the capsule of the crystalline lens, and *spirographin*[5] of the skeletal tissue of the worm Spirographis seem to act as such. Others on the contrary, such as *hyalin*[6] of the walls of hydatid cysts, *onuphin*[7] from the tubes of Onuphis tubicola, seem not to be compound proteids. The so-called *mucin of the holothures*,[8] and *chondrosin*[9] of the sponge, Chondrosia reniformis, and others may also be classed with the hyalogens. As the various bodies designated by KRUKENBERG as hyalogens are very dissimilar, it is not of much importance to arrange these in special groups.

Phosphoglycoproteids. This group includes the phosphorized glycoproteids. These compound proteids are decomposed by pepsin digestion and split off para- or pseudonuclein, similar to nucleoalbumins. They differ from the nucleoalbumins in that they yield a reducing substance on boiling with acids, and from the nucleoproteids in that they do not yield xanthin bases.

Only two phosphorized glycoproteids are known at the present time, namely, *ichthulin*, occurring in carp eggs and studied by WALTER[10] and which were considered as vitellin for a time. Ichthulin has the following composition: C 53.52; H 7.71; N 15.64; S 0.41; P 0.43; Fe 0.10%. In regard to solubilities it is similar to a globulin. WALTER has prepared a reducing substance from the paranuclein of ichthulin which gave a very crystalline combination with phenylhydrazin.

Another phosphoglycoproteid is *helicoproteid*, obtained by the author[11] from the glands of the snail Helix pomatia. It has the following composition: C 46.99; H 6.78; N 6.08; S 0.62; P 0.47%. It is converted into a gummy, lævorotatory carbohydrate, called *animal sinistrin*, by the action of alkalies. On boiling with an acid it yields a dextrorotatory, reducible substance.

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[1] Wien. Sitzungsber., Bd. 39, Abth. 2.

[2] Verh. d. physik.-med. Gesellsch. zu Würzburg, 1883; also Zeitschr. f. Biologie, Bd. 22.

[3] Krukenberg, Zeitschr. f. Biologie, Bd. 22.

[4] C. Th. Mörner, Zeitschr. f. physiol. Chem., Bd. 18.

[5] Krukenberg, Würzburg, Verhandl. 1883; also Zeitschr. f. Biologie. Bd. 22.

[6] A. Lücke, Arch. f. path. Anat., Bd. 19; also Krukenberg, Vergleichende physiol. Stud., Series 1 and 2, 1881.

[7] Schmiedeberg, Mitth. aus d. zool. Stat. zu Neapel, Bd. 3, 1882.

[8] Hilger, Pflüger's Archiv, Bd. 3.

[9] Krukenberg, Zeitschr. f. Biologie, Bd. 22.

[10] Zeitschr. f. physiol. Chem., Bd. 15.

[11] Pflüger's Archiv, Bd. 36.

Nucleoproteids. With this name we designate those compound proteids which yield true nucleins (see Chapter V) on pepsin digestion and those which yield, besides proteids, xanthin bases or so-called nuclein bases on boiling with dilute mineral acids.

The nucleoproteids seem to be widely diffused in the animal body. They occur chiefly in the cell nuclei, but they also often occur in the protoplasm. They may also pass into the animal fluids on the destruction of the cells, hence nucleoproteids have also been found in blood serum.

They may be considered as combinations of a proteid nucleus with a side chain, which KOSSEL[1] calls the PROSTETIC GROUP. This side chain, which contains the phosphorus, yields on the decomposition of certain nucleoproteids, such as from the yeast cell,[2] or from the pancreas,[3] besides nuclein bases also reducing substances, which form crystalline combinations with phenylhydrazin. It is still an open question as to the formation of reducing substances from other nucleoproteids. This prostetic group may be split off as nucleic acid (see Chapter V) by the action of alkalies. The nucleoproteids seem to be dissimilar according to the kind of nucleic acid split off because they yield differing relative amounts of the various xanthin bases.

The nucleoproteids are acids whose alkali compounds are soluble in water and which coagulate on heating (this is true at least for all genuine nucleoproteids investigated up to the present time). They may be precipitated from their alkali compounds by acetic acid, and the precipitate is more or less soluble in an excess of the acid. A confusion may occur here with nucleoalbumins and also with mucin substances. This confusion can be avoided by warming the body for some time on the water-bath with dilute sulphuric acid, and on cooling filtering and saturating the filtrate with ammonia and testing for xanthin bodies by an ammoniacal solution of silver nitrate. Any precipitate formed is examined more closely by the methods as given in Chapter V.

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[1] Verh. d. physiol. Gesellsch. zu Berlin, 1892–93, No. 1.

[2] A. Kossel, Du Bois-Reymond's Archiv, Physiol Abth., 1891.

[3] O. Hammarsten, Zeitschr. f. physiol. Chem., Bd. 19.

The properties of the various nucleoproteids are given more in detail in the various chapters which follow.

III. Albumoids or Albuminoids.

Under this name we collect into a special group all those protein bodies which cannot be placed in either of the other two groups, although they differ essentially among themselves and from a chemical standpoint do not show any radical difference from the true proteid bodies. The most important and abundant of the bodies belonging to this group are important constituents of the animal skeleton or the cutaneous structure. They occur as a rule in an insoluble state in the organism, and they are distinguished in most cases by a pronounced resistance to reagents which dissolve proteids or to chemical reagents in general.

The Keratin Group. Keratin is the chief constituent of the horny structure, of the epidermis, of hair, wool, of the nails, hoofs, horns, feathers, of tortoise-shell, etc., etc. Keratin is also found as neurokeratin (KÜHNE[1]) in the brain and nerves. The shell-membrane of the hen's egg seems also to consist of keratin.

It seems that there exist more than one keratin, and these form a special group of bodies. This fact, together with the difficulty in isolating the keratin from the tissues in a pure condition without a partial decomposition, is sufficient explanation for the variation in the elementary composition given below. As examples the analyses of a few tissues rich in keratin and of keratins are given as follows:

	C	H	N	S	O	
Human hair....	50.65	6.36	17.14	5.00	20.85	(v. LAER)[2]
Nail, ...	51.00	6.94	17.51	2.80	21.75	(MULDER)[3]
Neurokeratin.	56.11-58.45	7.26-8.02	11.46-14.32	1.63-2.24	.....	(KUHNE)[1]
Horn (average).	50.86	6.94	....	3.30	....	(HORBACZEWSKI)[4]
Tortoise-shell...	54.89	6.56	16.77	2.22	19.56	(MULDER)[3]
Shell-membrane	49.78	6.64	16.43	4.25	22.90	(LINDVALL)[5]

MOHR[6] has determined the quantity of sulphur in various keratin substances. The percentage varies from 2.6 to 5.3. Sulphur is at least in part in loose combination, and it is partly removed by the action of alkalies (as sulphides), or indeed in part by boiling with water. Combs of lead after long usage become black, and this is due to the action of the sulphur of the hair. On heating keratin with water in sealed tubes at a temperature of 150° to 200° C. it dissolves, with the elimination of sulphuretted hydrogen, forming a non-gelatinizing liquid which contains albumose (called *keratinose* by KRUKENBERG [1]) and peptone (?). Keratin is dissolved by alkalies, especially on heating, forming, besides alkali sulphides, albumoses and peptones (?).

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[1] Kühne and Ewald, Verh. d. naturhistor.-med. Vereins zu Heidelberg (N. F.), Bd. 1; also Kühne and Chittenden, Zeitschr. f. Biologie, Bd. 26.

[2] Annal. d. Chem. u. Pharm., Bd. 45.

[3] Versuch einer allgem. physiol. Chem. Braunschweig, 1844–51.

[4] See Drechsel in Ladenburg's Handwörterbuch d. Chem., Bd. 3.

[5] See Maly's Jahresbericht, 1881.

[6] Zeitschr. f. physiol. Chem., Bd. 20.

The decomposition products of keratins are moreover the same as the true proteids. On boiling with acids we obtain besides leucin and tyrosin, which occurs in relatively great amounts (1–5%), asparaginic acid[2] and glutamic acid,[3] ammonia, and sulphuretted hydrogen. HEDIN[4] has obtained a little lysin and considerable lysatinin from horn shavings. Besides these he obtained a sulphur compound whose hydrochloric-acid combination had the composition $C_{14}H_{28}N_4O_{12}SCl_4$, and another body which is perhaps identical with serin. There is no doubt that the keratins are derived from the proteids. DRECHSEL[5] is also of the opinion that in the keratin a part of the oxygen of the proteids is exchanged for sulphur, and a part of the leucin, or any other amido-acid, is exchanged for tyrosin. Keratin and proteids give the same decomposition products, with the exception that the former gives proportionally a greater quantity of tyrosin (1–5%). Among the sulphurized cleavage products of keratin EMMERLING[6] found *cystin*, and SUTER[7] *thio-lactic acid.* SUTER could not detect either cystin or cystein. Among the cleavage products obtained by the action of hydrochloric acid and tin chloride HEDIN[8] obtained a base which is probably identical with the base *arginin*, $C_6H_{14}N_4O_2$, isolated by SCHULZE and STEIGER[9] from lupin and malt acrospire.

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[1] Untersuch. über d. chem. Bau d. Eiweisskörper. Sitzungsber. d. Jenaischen Gesellsch. f. Med. u. Naturwissensch., 1886.

[2] Kreusler, Journ. f. prakt. Chem., Bd. 107.

[3] Horbaczewski, Sitzungsber. d. k. k. Wien. Akad. d. Wissensch., Bd. 80.

[4] Kgl. fysiogr. Sällsk. i Lund handlingar, Bd. 4; also Maly's Jahresber., Bd. 23.

[5] Drechsel in Ladenburg's Handwörterbuch d. Chem., Bd. 3.

[6] Chemiker-Zeitung, No. 80, 1894.

[7] Zeitschr. f. physiol. Chem., Bd. 20.

[8] *Ibid.*, Bd. 20.

[9] *Ibid.*, Bd. 11, S. 43.

Bodies occur in the animal kingdom which form intermediate bodies between coagulated albumin and keratin. C. TH. MÖRNER[1] has detected such a body in the tracheal cartilage, which forms a net-like basement membrane. This substance appears to be related to keratin on account of its solubilities and on the quantity of the sulphur (which turns lead black) it contains, while according to its solubility in gastric juice it must stand close to the proteids. Another substance, more similar to keratin, forms the horny layer in the gizzard of birds. According to J. HEDENIUS[2] this substance is insoluble in gastric or pancreatic juice and acts quite similar to keratin. It contains only 1% sulphur, and yields on decomposition only very little tyrosin besides considerable leucin.

Keratin is amorphous or takes the form of the tissues from which it was prepared. On heating it decomposes and generates an odor of burnt horn. It is insoluble in water, alcohol, or ether. On heating with water to 150°-200° C. it dissolves. It also dissolves gradually in caustic alkalies, especially on heating. It is not dissolved by artificial gastric juice or by trypsin solutions. Keratin gives the xanthoproteic reaction, as well as the reaction with MILLON's reagent, even though they are not always typical.

In the preparation of keratin a finely divided horny structure is treated first with boiling water, then consecutively with diluted acid, pepsin-hydrochloric acid, and alkaline trypsin solution, and, lastly, with water, alcohol, and ether.

Elastin occurs in the connective tissue of higher animals, sometimes in such large quantities that it forms a special tissue. It occurs most abundantly in the cervical ligament (ligamentum nuchæ).

Elastin is generally considered as a sulphur-free substance. According to the investigations of CHITTENDEN and HART,[3] it is a question whether or not elastin does not contain sulphur, which is removed by the action of the alkali in its preparation. H. SCHWARZ[4] has been able to prepare an elastin containing sulphur from the aorta by another method, and this sulphur can be removed by the action of alkalies, without changing the properties of the elastin. Elastin is hence perhaps a protein substance containing sulphur which exists only loosely combined. The most trustworthy analyses of elastin from the cervical ligament (Nos. 1 and 2) and from the aorta (No. 3) have given the following results:

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[1] Maly's Jahresber., Bd. 18.

[2] Skandinav. Arch. f. Physiol., Bd. 3.

[3] Zeitschr. f. Biologie, Bd. 25.

[4] Zeitschr. f. physiol. Chem., Bd. 18.

	C	H	N	S	O	
1.	54.32	6.99	16.75	....	21.94	(HORBACZEWSKI)[1]
2.	54 24	7.27	16.70	....	21.79	(CHITTENDEN and HART)[2]
3.	53.95	7.03	16.67	0.38	.....	(H. SCHWARZ)[3]

The splitting products of elastin are the same as for the true proteids with the difference that glycocoll but no aspartic and glutamic acids are obtained.[4] Tyrosin is only obtained in small quantities. SCHWARZ was able to detect lysatinin in the decomposition products, but not lysin positively. On putrefaction[5] no indol or phenol is obtained, but SCHWARZ, on the contrary, obtained indol, skatol, benzol, and phenols, but no methylmercaptan, on fusing aorta-elastin with caustic potash. On heating with water in closed vessels, on boiling with dilute acids, or by the action of proteolytic enzymes, the elastin dissolves and splits into two chief products, called by HORBACZEWSKI *hemielastin* and *elastinpeptone.* According to CHITTENDEN and HART, these products correspond to two albumoses designated by them *protoelastose* and *deutero-elastose.* The first is soluble in cold water and separates on heating, and its solution is precipitated by mineral acid as well as by acetic acid and potassium ferrocyanide. The watery solution of the other does not become cloudy on heating, and is not precipitated by the above-mentioned reagents.

Pure dry elastin is a yellowish-white powder; in the moist state it appears like yellowish-white threads or membranes. It is insoluble in water, alcohol, or ether, and shows a resistance against the action of chemical reagents. It is not dissolved by strong caustic alkalies at the ordinary temperature, and only slowly at the boiling temperature. It is very slowly attacked by cold concentrated sulphuric acid, and it is relatively easily dissolved on warming with strong nitric acid. Elastins of differing origins act differently with cold concentrated hydrochloric acid; for instance, elastin from the aorta dissolves readily therein, while elastin from the ligamentum nuchæ, at least from old animals, dissolves with difficulty. Elastin is more readily dissolved by warm concentrated hydrochloric acid. It responds to the xanthoproteic reaction and with MILLON'S reagent.

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[1] Zeitschr. f. physiol. Chem., Bd. 6.

[2] Zeitschr. f. Biologie, Bd. 25.

[3] Zeitschr. f. physiol. Chem., Bd. 18.

[4] See Drechsel in Ladenburg's Handwörterbuch d. Chem., Bd. 3, and Horbaczewski, Monatshefte f. Chem., Bd. 6.

[5] Wälchli, Journ. f. prakt. Chem., Bd. 17.

On account of its great resistance to chemical reagents, elastin may be prepared (best from the ligamentum nuchæ) in the following way: First boil with water, then with 1% caustic potash, then again with water, and lastly with acetic acid. The residue is treated with cold 5% hydrochloric acid for twenty-four hours, carefully washed with water, boiled again with water, and then treated with alcohol and ether.

SCHWATZ first incompletely digested the tissues with pepsin, washed first with soda solution and then with water, and boiled lastly with water until the elastic substance was dissolved away. The dried and powdered substance is again digested with gastric juice and treated as above, and then boiled with water until the contaminating reticulin-like substance is completely removed.

Collagen, or gelatine-forming substance, occurs very extensively in the animal kingdom. The flesh of cephalopods is claimed to contain collagen.[1] Collagen is the chief constituent of the fibrils of the connective tissue and (as ossein) of the organic substances of the bony structure. It also occurs in the cartilaginous tissues as chief constituent, but it is here mixed with other substances, producing what was formerly called chondrigen. Collagen from different tissues has not quite the same composition, and probably there are several varieties of collagen.

By continuously boiling with water (more easily in the presence of a little acid) collagen is converted into gelatine. HOFMEISTER[2] found that gelatine, on being heated to 130° C., is again transformed into collagen; and this last may be considered as the anhydride of gelatine. Collagen and gelatine have about the same composition:

	C	H	N	S + O	
Collagen................	50.75	6.47	17.86	24.92	(HOFMEISTER)[2]
Gelatine (from hartshorn).	49.31	6.55	18.37	25.77	(MULDER)[3]
Gelatine (from bones).....	50.00	6.50	17.50	26.00	(FREMY)[4]
Purified Gelatine.........	50.14	6.69	18.12	....	(PAAL)[5]

The gelatine contains about 0.6% sulphur, which probably belongs to the gelatine and does not exist there as an impurity from the proteids.

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[1] Hoppe-Seyler, Physiol. Chem. Berlin, 1877–81. S. 97.

[2] Zeitschr. f. physiol. Chem., Bd. 2.

[3] Annal. d. Chem. u. Pharm., Bd. 45.

[4] Jahresber. d. Chem., 1854.

[5] Ber. d. deutsch. chem. Gesellsch., Bd. 25, S. 1208.

The decomposition products of collagen are the same as those of gelatine. Gelatine under similar conditions as the proteids yields amido-acids, such as leucin, aspartic and glutamic acids, but no tyrosin, which is especially important. It yields, on the contrary, large quantities of glycocoll, to which the name gelatine sugar is given on account of its sweet taste. Lysin and lysatinin have also been obtained from gelatine by DRECHSEL and E. FISCHER.[1] On putrefaction gelatine yields neither tyrosin, indol nor skatol,[2] in which it differs from the proteids. Still the aromatic group is not absent in gelatine, and it acts like the oxidized proteid, the oxyprotsulphonic acid, yielding benzoic acid (MALY[3]).

Collagen is insoluble in water, salt solutions, dilute acids, and alkalies, but it swells up in dilute acids. By continuous boiling with water it is converted into gelatine. It is dissolved by the gastric juice and also by the pancreatic juice (trypsin solution) when it has previously been treated with acid or heated with water above + 70° C.[4] By the action of ferrous sulphate, corrosive sublimate, or tannic acid, collagen shrinks greatly. Collagen treated by these bodies does not putrefy, and tannic acid is therefore of great importance in the preparation of leather.

Gelatine or glutin is colorless, amorphous, and transparent in thin layers. It swells in cold water without dissolving. It dissolves in warm water, forming a sticky liquid, which solidifies on cooling when sufficiently concentrated. The quantity of ash contained in gelatine is of the greatest importance in the gelatinization of gelatine solutions, as shown by O. NASSE and A. KRÜGER,[5] namely, a diminished quantity of ash diminishes the gelatinization power.

Gelatine solutions are not precipitated on boiling, neither by mineral acids, acetic acid, alum, lead acetate, nor mineral salts in general. A gelatine solution acidified with acetic acid may be precipitated by potassium ferrocyanide on carefully adding the reagent, but on the addition of too much potassium ferrocyanide the liquid remains clear. Gelatine solutions are precipitated by tannic acid in the presence of salt; by acetic acid and common salt in substance; mercuric chloride in the presence of HCl and NaCl; metaphosphoric acid, phosphomolybdic acid in the presence of acid; and lastly by alcohol, especially when neutral salts are present. Gelatine solutions do not diffuse. Gelatine gives the biuret reaction, but not ADAMKIEWICZ'S. It gives MILLON'S reaction and the xanthoproteic acid reaction so faintly that it probably occurs from an impurity consisting of proteids.

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[1] See Drechsel, Der Abbau der Eiweisskörper. Du Bois-Reymond's Archiv, 1891.

[2] See literature on the cleavage products of gelatine: Drechsel in Ladenburg's Handwörterbuch, Bd. 3.

[3] Monatshefte f. Chem., Bd. 10.

[4] Kühne and Ewald, Verh. d. naturhist. med. Vereins in Heidelberg, 1877, Bd. 1.

[5] See Maly's Jahresber., Bd. 19, S. 29.

By continuous boiling with water glutin is converted into a non-gelatinizing modification called β-glutin by NASSE. According to NASSE and KRÜGER the specific rotatory power is hereby reduced from $-167°.5$ to about $-136°$. On long-continued boiling with water, especially in the presence of dilute acids, also in the gastric or tryptic digestion, the gelatine is transformed into gelatine albumoses, so-called *gelatoses* and *gelatine peptones*, which diffuse more or less readily.

According to HOFMEISTER[1] two new substances, *semiglutin* and *hemicollin*, are formed. The former is insoluble in alcohol of 70–80% and is precipitated by platinum chloride. The latter, which is not precipitated by platinum chloride, is soluble in alcohol. CHITTENDEN and SOLLEY[2] have obtained in the peptic and tryptic digestion a *proto-* and a *deuterogelatose*, besides some true peptone. The elementary composition of the gelatoses does not essentially differ from that of the gelatine. PAAL[3] has prepared gelatine peptone hydrochlorides from gelatine by the action of dilute hydrochloric acid. Some of these salts are soluble in ethyl and methyl alcohol, and others insoluble therein. The peptones obtained from these salts contain less carbon and more hydrogen than the glutin from which they originated, showing that hydration has taken place. The molecular weight of the gelatine peptone as determined by PAAL by RAOULT'S method was 200 to 352, while that for gelatine was 878 to 960.

Collagen may be obtained from bones by extracting them with hydrochloric acid (which dissolves the earthy phosphates) and then carefully removing the acid with water. It may be obtained from tendons by extracting with lime-water or dilute alkali (which dissolve the proteids and mucin) and then thoroughly washing with water. Gelatine is obtained by boiling collagen with water. The finest commercial gelatine always contains a little proteid, which may be removed by allowing the finely divided gelatine to swell up in water and thoroughly extracting with large quantities of fresh water. Then dissolve in warm water and precipitate with alcohol.

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[1] Zeitschr. f. physiol. Chem., Bd. 2.

[2] Journ. of physiol., Vol. 12.

[3] Ber. d. deutsch. chem. Gesellsch., Bd. 25.

Chondrin or cartilage gelatine is only a mixture of glutin with the specific constituents of the cartilage and their transformation products.

Reticulin. The reticular tissues of the lymphatic glands contain a variety of fibres which have also been found by MALL[1] in the spleen, intestinal mucosa, liver, kidneys, and lungs. These fibres consist of a special substance, reticulin, investigated by SIEGFRIED.[2]

Reticulin has the following composition: C 52.88; H 6.97; N 15.63; S 1.88; P 0.34; ash 2.27. The phosphorus occurs in organic combination. It yields no tyrosin on splitting with hydrochloric acid. It yields, on the contrary, sulphuretted hydrogen, ammonia, lysin, lysatinin, and amido-valerianic acid. On continuous boiling with water, or more readily with dilute alkalies, reticulin is converted into a body which is precipitated by acetic acid, and at the same time phosphorus is split off.

Reticulin is insoluble in water, alcohol, ether, lime-water, sodium carbonate, and dilute mineral acids. It is dissolved, after several weeks, on standing with caustic soda at the ordinary temperature. Pepsin hydrochloric acid or trypsin do not dissolve it. Reticulin responds to the biuret, xanthoproteic, and ADAMKIEWICZ'S reactions, but not with MILLON'S reagent.

It may be prepared as follows, according to SIEGFRIED: Digest intestinal mucosa with trypsin and alkali. Wash the residue, extract with ether, and digest again with trypsin and then treat with alcohol and ether. On careful boiling with water the collagen present either as contamination or as a combination with reticulin is removed. The thoroughly dried residue consists of reticulin.

Skeletins are a number of nitrogenized substances which form the skeletal tissue of various classes of invertebrates so designated by KRUKENBERG.[3] These substances are *chitin*, *spongin*, *conchiolin*, *cornein*, and *fibroin* (silk). Of these chitin does not belong to the protein substances, and fibroin (silk) is hardly to be classed as a skeletin. Only those so-called skeletins will be given that actually belong to the protein group.

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[1] Abhandl. d. math.-phys. Klasse d. kgl. sächs. Gesellsch. d. Wiss., 1891.

[2] Ueber die chemischen Eigenschaften des reticulirten Gewebes. Inaugural dissertation. Leipzig, 1892.

[3] Grundzüge einer vergl. Physiol. d. thier. Gerüstsubst. Heidelberg, 1885.

Spongin forms the chief mass of the ordinary sponge. It gives no gelatine on boiling with acids, but yields leucin and glycocoll and no tyrosin. ZALOCOSTAS[1] claims to have found tyrosin and also butalanin and glycalanin ($C_5H_{12}N_2O_4$). **Conchiolin** is found in the shells of mussels and snails and also in the egg-shells of these animals. It yields leucin but no tyrosin. The **Byssus** contains a substance, closely related to conchiolin, which is soluble with difficulty. **Cornein** forms the axial system of the Antipathes and Gorgonia. It gives leucin and a crystallizable substance, *cornicrystallin* (KRUKENBERG). **Fibroin** and **Sericin** are the two chief constituents of raw silk. By the action of superheated water the sericin dissolves and gelatinizes on cooling (silk gelatine), while the more difficultly soluble fibroin remains undissolved in the shape of the original fibre. On boiling with acid the fibroin yields alanin (WEYL[2]), glycocoll, and a great deal (5–8%) of tyrosin. Fibroin is dissolved in cold concentrated hydrochloric acid with the expulsion of 1% nitrogen as ammonia, and it is converted into another, nearly related substance called *sericoin* (WEYL). Sericin yields no glycocoll, but leucin and a crystallizable substance called *serin* (amidoethylenlactic acid). The composition of the above-mentioned bodies is as follows:

	C	H	N	S	O	
Conchiolin (from snail-eggs)	50.92	6.88	17.86	0.31	24.34	(KRUKENBERG)[3]
Spongin	46.50	6.30	16.20	0.5	27.50	(CROOCKEWITT)[4]
"	48.75	6.35	16.40	....	.....	(POSSELT)[5]
Cornein	48.96	5.90	16.81	....	28.33	(KRUKENBERG)[6]
Fibroin	48.23	6.27	18.31	....	27.19	(CRAMER)[7]
"	48.30	6.50	19.20	....	26.00	(VIGNON)[8]
Sericin	44.32	6.18	18.30	....	30.20	(CRAMER)

Amyloid, so called by VIRCHOW, is a protein substance appearing under pathological conditions in the internal organs, such as the spleen, liver, and kidneys, as infiltrations; and in serous membranes as granules with concentric layers. It probably also occurs as a constituent of certain prostate calculi. Amyloid has not been obtained pure, therefore its composition cannot be given with certainty. FRIEDREICH and KEKULÉ[9] found C 53.6; H 7.0; N 15.0; and S + O 24.4%. KÜHNE and RUDNEFF[10] found 1.3% sulphur. Amyloid is not related to the carbohydrates in the ordinary sense, and on boiling with acids it gives neither glucose nor any other reducing substance. On the contrary, it yields leucin and tyrosin. According to KRAWKOW,[1] amyloid yields a residue similar to chitin on boiling with strong caustic alkali.

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[1] Compt. rend., Tome 107.

[2] Ber. d. deutsch. chem. Gesellsch., Bd. 21.

[3] *Ibid.*, Bd. 18, and Zeitschr. f. Biologie, Bd. 22.

[4] Annal. d. Chem. u. Pharm., Bd. 48.

[5] *Ibid.*, Bd. 45.

[6] Ber. d. deutsch. chem. Gesellsch., Bd. 17.

[7] Journ. f. prakt. Chem., Bd. 96.

[8] Compt. rend., Tome 115.

[9] Virchow's Archiv, Bd. 16.

[10] *Ibid.*, Bd. 33.

It is insoluble in water, alcohol, ether, dilute hydrochloric acid, and acetic acid. It is dissolved in concentrated hydrochloric acid or caustic alkali, and is converted into acid or alkali albuminates depending upon the agents employed. According to KOSTJURIN,[2] amyloid is dissolved by the gastric juice, which is the reverse of older theories. A. TSCHERMAK[3] found that the amyloid from the liver and spleen was readily soluble in alkalies, less soluble in organic acids and mineral acid, as well as by peptic or tryptic digestion or by heating in sealed tubes with water. First soluble, unchanged amyloid is formed, which is then transformed into albuminates, albumoses, and peptones. All these products give the same color reactions as the mother-substance. TSCHERMAK considers amyloid as a coagulated proteid. Amyloid gives the xanthoproteic reaction and the reactions of MILLON and ADAMKIEWICZ. Its most important property is its behavior with certain coloring matters. It is colored reddish brown or a dingy violet by iodine; a violet or blue by iodine and sulphuric acid; red by methylaniline iodide, especially on the addition of acetic acid; and red by aniline green.

Amyloid is prepared by extracting the tissue with cold and then boiling water, afterwards with alcohol and ether. After boiling with alcohol containing hydrochloric acid and digesting with gastric juice, that which is insoluble is considered as amyloid. As the amyloid may be dissolved by the gastric juice (KOSTJURIN), the utility of this method seems doubtful.

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[1] Centralbl. f. d. med. Wissensch, 1892.

[2] Wien. med. Jahrbücher, 1886. Cit. from Maly's Jahresber., Bd. 16, S. 32.

[3] Zeitschr. f. physiol. Chem., Bd. 20.

CHAPTER III.

THE CARBOHYDRATES.

We designate with this name bodies which occur especially abundant in the plant kingdom. As the protein bodies form the chief portion of the solids in animal tissues, so the carbohydrates form the chief portion of the dry substance of the plant structure. They occur in the animal kingdom only in proportionately small quantities either free or in combinations with more complex molecules, forming compound proteids. Carbohydrates are of extraordinarily great importance as food for both man and animals.

The carbohydrates contain *carbon*, *hydrogen*, and *oxygen*. The last two elements occur in the same proportion as they do in water, namely, 2 : 1, and this is the reason why the name carbohydrates has been given to them. This name is not quite pertinent, if strictly considered; because even though we have bodies, such as acetic acid and lactic, which are not carbohydrates and still have their oxygen and hydrogen in the relationship to form water, nevertheless we also have sugars (rhamnose, $C_6H_{12}O_5$) which have these two elements in another proportion. Heretofore it was thought possible to characterize as carbohydrates those bodies which contained 6 atoms of carbon, or a multiple, in the molecule, but this is not considered valid at the present time. We have true carbohydrates containing less than 6 and also those containing 7, 8, and 9 carbon atoms in the molecule. The carbohydrates have no properties or characteristics in general which differentiate them from other bodies; on the contrary, the various carbohydrates are in many cases very different in their external properties. Under these circumstances it is very difficult to give a positive definition of carbohydrates.

From a chemical standpoint we can say that all carbohydrates are aldehyde or ketone derivatives of hexatomic alcohols. The simplest carbohydrates, the simple sugars or monosaccharides, are either aldehyde or ketone derivatives of these alcohols, and the more complex carbohydrates seem to be derived from these by the formation of anhydrides. It is a fact that the more complex carbohydrates yield two or even more molecules of the simple sugars when made to undergo hydrolytic splitting.

The carbohydrates are generally divided into three chief groups, namely, *monosaccharides*, *disaccharides*, and *polysaccharides*.

Our knowledge of the carbohydrates and their structural relationships have been very much extended by the pioneering investigations of KILIANI[1] and especially those of E. FISCHER.[2]

As the carbohydrates occur chiefly in the plant kingdom it is naturally not the place here to give a complete discussion of the numerous carbohydrates known up to the present time. According to the plan of this work it is only possible to give a short review of those carbohydrates which occur in the animal kingdom or are of special importance as food for man and animals.

Monosaccharides.

All varieties of sugars, the monosaccharides as well as disaccharides, are characterized by the termination "ose," to which a root is added signifying their origin or other relations. According to the number of carbon atoms contained in the molecule the monosaccharides are divided into *tioses*, *tetroses*, *pentoses*, *hexoses*, *heptoses*, and so on.

All monosaccharides are either aldehydes or ketones of hexatomic alcohols. The first are termed *aldoses* and the other *ketoses*. Ordinary glucose is an aldose, while ordinary fruit-sugar (fructose) is a ketose. The difference may be shown by the structural formula of these two varieties of sugar:

$$\text{Glucose} = CH_2(OH).CH(OH).CH(OH).CH(OH).CH(OH).CHO;$$
$$\text{Fructose} = CH_2(OH).CH(OH).CH(OH).CH(OH).CO.CH_2(OH).$$

A difference is also observed on oxidation. The aldoses can be converted into oxyacids having the same quantity of carbon, while the ketoses yield acids having less carbon. On mild oxidation the aldoses yield monobasic oxyacids and dibasic acids on more energetic oxidation. Thus ordinary glucose yields gluconic acid in the first case and saccharic acid in the second.

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[1] Ber. d. deutsch. chem. Gesellsch., Bdd. 18, 19, and 20.

[2] See E. Fischer's lecture: "Synthesen in der Zuckergruppe," Ber. d. deutsch. chem. Gesellsch., Bd. 23, S. 2114. An excellent work on Carbohydrates is Tollen's "Kurzes Handbuch der Kohlehydrate," Breslau, 1888, which gives a complete review of the literature.

Gluconic acid $= CH_2(OH).[CH(OH)]_4.COOH$;
Saccharic acid $= COOH.[CH(OH)]_4.COOH$.

The monobasic oxyacids are of the greatest importance in the artificial formation of the monosaccharides. These acids, as lactones, can be converted into their respective aldehydes (corresponding to the sugars) by the action of nascent hydrogen. On the other hand they may be transformed into stereo-isomeric acids on heating with chinolin, pyridin, etc., and the stereo-isomeric sugars may be obtained from these by reduction.

Numerous isomers occur among the monosaccharides, and especially in the hexose group. In certain cases, as for instance in glucose and fructose, we are dealing with a different constitution (aldoses and ketoses), but in most cases we have stereo-isomerism due to the presence of asymmetric carbon atoms.

The monosaccharides are converted into the corresponding alcohols by nascent hydrogen. Thus ARABINOSE, which is a pentose, $C_5H_{10}O_5$, is transformed into the pentatomic alcohol, ARABIT, $C_5H_{12}O_5$. The three hexoses, GLUCOSE, FRUCTOSE, and GALACTOSE, $C_6H_{12}O_6$, are transformed into the corresponding three hexatomic alcohols, SORBITE, MANNITE, and DULCITE, $C_6H_{14}O_6$. Inversely, the corresponding sugars may be prepared from their alcohols by careful oxidation.

Similar to the ordinary aldehydes and ketones the sugars may be made to take up hydrocyanic acid. Cyanhydrines are thus formed. These addition products are of special interest in that they make the artificial preparation possible of sugars rich in carbon from sugars poor in carbon.

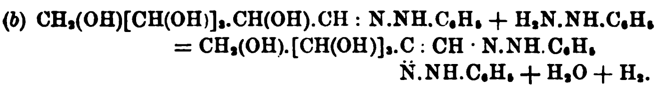
As example, if we start from glucose we obtain glucocyanhydrin on the addition of hydrocyanic acid: $CH_2(OH).[CH(OH)]_4.COH + HCN = CH_2(OH).[CH(OH)]_4.CH(OH).CN$. On the saponification of glucocyanhydrin the corresponding oxyacid is formed: $CH_2(OH).[CH(OH)]_4.CH(OH).CN + 2H_2O = CH_2(OH).[CH(OH)]_4.CH(OH).COOH + NH_3$. By the action of nascent hydrogen on the lactone of this acid we obtain glucoheptose, $C_7H_{14}O_7$.

The monosaccharides give the corresponding oximes with hydroxylamin; thus glucose yields glucosoxime, $CH_2(OH).[CH(OH)]_4.CH : N.OH$. These combinations are of importance on account of the fact, as found by WOHL,[1] that they are the starting-point in the building up of varieties of sugars, namely, the preparation of sugars poor in carbon from those rich in carbon.

The monosaccharides are strong reducing bodies, similar to the aldehydes. They reduce metallic silver from ammoniacal silver solutions, and also several metallic oxides, such as copper, bismuth, and mercury oxides, on warming their alkaline solutions. This property is of the greatest importance in their detection and quantitative estimation.

The behavior of the sugars to phenylhydrazin acetate is of special importance. Their watery solutions first yield HYDRAZONES with phenylhydrazin acetate, and then OSAZONES on lengthy warming in the water-bath. The reaction takes place as follows:

(a) $CH_2(OH).[CH(OH)]_3.CH(OH).CHO + H_2N.NH.C_6H_5$
$= CH_2(OH).[CH(OH)]_3.CH(OH)CH : N.NH.C_6H_5 + H_2O.$
Phenylglucoshydrazon.



$$\begin{array}{l}(b)\ CH_2(OH)[CH(OH)]_3.CH(OH).CH : N.NH.C_6H_5 + H_2N.NH.C_6H_5 \\ \quad = CH_2(OH).[CH(OH)]_3.C : CH \cdot N.NH.C_6H_5 \\ \qquad\qquad\qquad\qquad\qquad \ddot{N}.NH.C_6H_5 + H_2O + H_2.\end{array}$$

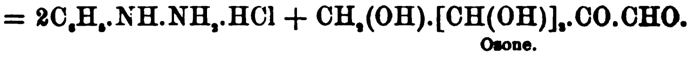
Phenylglucosazon.

The hydrogen is not evolved, but acts on a second molecule of phenylhydrazon and splits it into anilin and ammonia:

$$H_2N.NH.C_6H_5 + H_2 = H_2N.C_6H_5 + NH_3.$$

The osazones are yellow crystalline combinations, which differ from each other in melting-point, solubility, and optical properties and hence have received great importance in the characterization of certain sugars. They have also become of extraordinarily great importance in the study of the carbohydrates for other reasons. Thus they are very good means of precipitating sugars from solution in which they occur mixed with other bodies, and they are of the greatest importance in the artificial preparation of sugars.

On splitting, by the short action of gentle heat and fuming hydrochloric acid, the osazones yield phenylhydrazin hydrochloride and so-called OSONES, bodies which are ketoaldehydes:



$$\begin{array}{l}CH_2(OH).[CH(OH)]_3.C.CH : N.NH.C_6H_5 \\ \qquad\qquad\qquad\qquad\quad \ddot{N}.NH.C_6H_5 \quad + 2H_2O + 2HCl \\ = 2C_6H_5.NH.NH_2.HCl + CH_2(OH).[CH(OH)]_3.CO.CHO.\end{array}$$

Osone.

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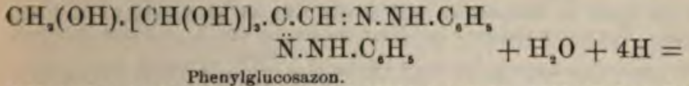
[1] Ber. d. deutsch. chem. Gesellsch., Bd. 26, S. 730.

The ketoses are obtained from the osones by reduction with zinc dust and acetic acid:

$$CH_2(OH).[CH(OH)]_3.CO.CHO + 2H$$
$$= CH_2(OH).[CH(OH)]_3.CO.CH_2(OH).$$

If we start with an aldose, we do not get the same sugar back again, but an isomere ketose, and in this way we can convert glucose into fructose.

We can also pass from the osazones to the corresponding sugars (ketoses) in other ways, namely, by direct reduction of the osazones with acetic acid and zinc dust. The corresponding osamin is first formed, and then on treating with nitrous acid a ketose is obtained:



$$CH_2(OH).[CH(OH)]_3.\underset{\ddot{N}.NH.C_6H_5}{C}.CH:N.NH.C_6H_5 + H_2O + 4H =$$

Phenylglucosazon.

$$CH_2(OH).[CH(OH)]_3.CO.CH_2(NH_2)+C_6H_5.NH.NH_2+C_6H_5.NH_2$$

Isoglucosamin.

and

$$CH_2(OH).[CH(OH)]_3.CO.CH_2(NH_2) + HNO_2$$
$$= CH_2(OH).[CH(OH)]_3.CO.CH_2(OH) + N_2 + H_2O.$$

Fructose.

From what has been stated we see that there are various ways of preparing sugars artificially. They may be prepared (1) by the careful oxidation of the related alcohols; (2) reduction of the corresponding monobasic oxyacids; (3) splitting of the osazone with hydrochloric acid and a reduction of the osone; (4) direct reduction of the osazone and treating the osamin with nitrous acid; (5) syntheses from combinations poor in carbon (see syntheses of the hexoses).

The monosaccharides are colorless and odorless bodies, neutral in reaction, with a sweet taste, readily soluble in water, generally soluble with difficulty in absolute alcohol, and insoluble in ether, and some of which crystallize well in the pure state. They are optically active, some lævorotatory and others dextrorotatory; but there are also optically inactive modifications (racemic), which are formed from two optically opposed components.

We designate the optical activity of the carbohydrates with the letter l- for lævogyrate, d- for dextrogyrate, and i- for inactive. These are only partly useful. Thus dextrorotatory glucose is designated d-glucose, lævorotatory l-glucose, and the inactive i-glucose. EMIL FISCHER has used these signs in another sense. He designates by these signs the homogeneousness of the various kinds of sugars instead of their optical activity. For example, he does not designate the lævorotatory fructose, l-fructose, but d-fructose, showing its close relation to dextrorotatory d-glucose. This designation is generally accepted, and the above-mentioned signs only show the optical properties in a few cases.

Specific rotation means the rotation in degrees produced by 1 gm. substance dissolved in 1 cc. liquid placed in a tube 1 d. cm. long. The reading is ordinarily made at + 20° C. and with a homogeneous sodium light. The sp. rotation with this light is represented by $\alpha(D)$, and is expressed by the following formula: $\alpha(D) = \pm \frac{a}{p.1}$, in which a represents the reading of degrees, 1 the length of the tube in decimetres, and p the weight of substance in 1 cc. of the liquid. Inversely the per cent P of substance can be calculated, when the specific rotation is known, by the formula $P = \frac{100a}{s.1}$, in which s represents the known specific rotation.

A freshly prepared sugar solution often shows another rotation form, when it is allowed to stand for some time. If the rotation gradually diminishes, this is called birotation, while a gradual increase in the rotation is called half-rotation. The birotation and half-rotation may be immediately abolished by the addition of very little ammonia (1 p. m.). C. SCHULTZE and TOLLINS.[1]

Many monosaccharides, but not all, ferment with yeast, and it has been shown that only those varieties of sugar containing 3, 6, or 9 atoms of carbon in the molecule are fermentable with yeast. Still amongst the hexoses we find exceptions, namely, a few artificially prepared hexoses do not ferment with yeast. Various kinds of schizomycetes cause a different fermentation, such as lactic and butyric acid fermentation and mucilaginous fermentation.

The simple varieties of sugar occur in part in nature as such already formed, which is the case with both of the very important sugars, grape-sugar and fructose. They also occur in great abundance in nature as more complex carbohydrates (di- and polysaccharides); also as ester combinations with different substances, as so-called glucosides.

Among the groups of monosaccharides known at the present time, those containing less than five and more than six carbon atoms in the molecule have no great importance in zoo-chemistry, although they are of high scientific interest. Of the other two groups the hexoses are of the greatest importance, because in the past only those carbohydrates with six carbon atoms were considered as true carbohydrates. As the pentoses have been the subject of zoo-chemical investigations of late, they will also be given in short.

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[1] Annal. d. Chem. u. Pharm., Bd. 271.

Pentoses ($C_5H_{10}O_5$).

As a rule the pentoses do not occur as such in nature, but are formed in the hydrolytic splitting of several complex carbohydrates, the so-called pentosanes, especially on boiling gums with dilute mineral acids. They exist very widely distributed in the plant kingdom, and are especially of great importance in the building up of certain plant constituents. They have only thus far been found in exceptional cases in animals. SALKOWSKI and JASTROWITZ[1] have found a pentose in the urine of those addicted to the morphine habit. A pentose has been found by the author[2] amongst the cleavage products of a nucleoproteid from the pancreas.

The pentoses seem to be of importance as food for herbivorous animals. SALKOWSKI[3] and CREMER[4] have shown that the pentoses xylose, arabinose, and rhamnose are absorbed by rabbits and hens, and that these animals utilize the pentoses, and even form glycogen therefrom. The pentoses seem to be absorbed by human beings, but the views in regard to their assimilation are somewhat disputed.[5]

The pentoses are non-fermentable, reducible aldoses. On heating with sulphuric or hydrochloric acids they yield furfurol, but no levulinic acid. The furfurol passing over on distilling with hydrochloric acid may not only be used in the detection (with aniline acetate paper which is colored red with furfurol), but also in the quantitative estimation of pentoses (or pentosanes). On warming with hydrochloric acid containing phloroglucin a beautiful red solution is the result, and this solution gives a sharply-defined absorption band on the right of the sodium line. The most important pentoses are ARABINOSE and XYLOSE.

Arabinose (dextro-rotatory arabinose, pectin sugar) is obtained on boiling gum arabic or cherry-gum with 2% sulphuric acid. It crystallizes, has a sweet taste, melts at about 160°, and is strongly dextro-rotatory. Its osazon melts at 157–158° C. The artificially prepared lævogyrate as well as the optically inactive arabinose are known.

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[1] Centralbl. f. d. med. Wissensch., 1892, S. 337 and 593.

[2] Zeitschr. f. physiol. Chem., Bd. 19.

[3] Centralbl. f. d. med. Wissensch., 1892, S. 337 and 593.

[4] Zeitschr. f. Biologie, Bd. 29.

[5] See Ebstein, Virchow's Arch., Bd. 129, and Cremer, Zeitschr. f. Biologie, Bd. 29.

Xylose (wood sugar). This body is obtained with the previous stereo-isomeric pentose on boiling wood gums with dilute acids. It crystallizes, is feebly dextrogyrate, and gives an osazon, which melts at about 160° C.

Amongst the pentoses we have *ribose*, obtained on the reduction of the lactone of ribonic acid, which is produced from arobonic acid. *Rhamnose*, which used to be called isodulcite, is a methylpentose, $C_6H_{12}O_5$, and is obtained from different glucosides (quercitin, xanthorhamnin, etc.).

Hexoses ($C_6H_{12}O_6$).

The most important and best-known simple sugars belong to this group, and the remaining bodies considered as carbohydrates (with the exception of arabinose and inosite) are anhydrides of this group. Certain hexoses, such as dextrose and fructose, occur in nature already formed, while others are produced by the hydrolytic splitting of other more complicated carbohydrates or glucosides. Others, such as mannose or galactose, are formed by the hydrolytic cleavage of natural products; while some, on the contrary, such as gulose, talose, and others, are obtained only by artificial means.

All hexoses, as also their anhydrides, yield levulinic acid, $C_5H_8O_3$, besides formic acid and humus substances, on boiling with dilute mineral acids. Some of the hexoses are fermentable with yeast, while the artificially prepared hexoses do not, or at least only with great difficulty and incompletely.

Some hexoses are aldoses, while others are ketoses. Belonging to the first group we have MANNOSE, GLUCOSE, GULOSE, GALACTOSE, and TALOSE, and to the other FRUCTOSE, and possibly also SORBINOSE. We differentiate also between the d, l, and i modifications, for instance, d-, l-, and i-glucose; hence the number of isomers is very great.

The most important syntheses of the carbohydrates have been made by E. FISCHER and his pupils chiefly within the members of the hexose group. A short summary of the syntheses of hexoses is given below.

The first artificial preparation of glucose was made by BUTLEROW.[1] On treating trioxymethylen, a polymer of formaldehyde, with lime-water he obtained a faintly sweetish syrup called *methylenitan*. LOEW[1] later obtained about the same product on the condensation of formaldehyde in the presence of bases, and he called this product formose. E. FISCHER[2] has shown that this formose syrup consists of a mixture of a nonfermentable sugar, *formose*, and a fermentable sugar, *α-acrose*. This last-mentioned hexose is the starting-point for further syntheses.

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[1] Ann. d. Chem. u. Pharm., Bd. 120 · Compt. rend., Tome 53.

The name α-acrose has been given to these bodies because they are obtained from acrolein bromide by the action of bases (FISCHER). They are also obtained admixed with *β-acrose* on the oxidation of glycerin with bromine in the presence of sodium carbonate, and treating the resulting mixture of glycerin. aldehyde, and dioxyaceton, $CH_2(OH).CH(OH).CHO$ and $CH_2(OH).CO.CH_2(OH)$ with alkalies. A condensation takes place with the formation of hexoses.

α-acrose may be isolated from the above mixture and obtained pure by first converting it into its osazon and then retransforming this into the sugar. α-acrose is identical with i-fructose. With yeast one-half, the lævogyrate d-fructose ferments, while the dextrogyrate l-fructose remains. The i- and l-fructose may be prepared in this way.

On the reduction of α-acrose we obtain α-acrit, which is identical with i-mannite. On oxidation of i-mannite we obtain i-mannose, from which only l-mannose remains on fermentation. On further oxidation of i-mannose it yields i-mannonic acid. The two active mannonic acids may be separated from each other by the fractional crystallization of their strychnin or morphin salts. The two corresponding mannoses may be obtained from these two acids, d- and l-mannonic acids, by reduction.

d-fructose is obtained from d-mannose by the method given on page 63, using the osazon as an intermediate step. The d- and l-mannonic acids are partly converted into d- and l-gluconic acid on heating with chinolin, and d- or l-glucose is obtained on the reduction of these acids. l-glucose is best prepared from l-arabinose by means of the cyanhydrin, reaction, using l-gluconic acid as the intermediate step. The combination of l- and d-gluconic acid, forming i-gluconic acid, yields i-glucose on reduction.

The artificial preparation of sugars by means of condensation of formaldehyde has received special interest because, according to BAEYER's assimilation hypothesis of plants, formaldehyde is first formed by the reduction of carbon dioxide, and the sugars are produced by the condensation of this formaldehyde. BOKORNY[3] has shown, by special experiments on algæ Spirogyra, that formaldehyde sodium sulphite was split by the living algæ cells. The formaldehyde set free is immediately condensed to carbohydrate and precipitated as starch.

Among the hexoses known at the present time only dextrose, fructose, and galactose are really of physiological chemical interest; therefore the other hexoses will only be incidentally mentioned.

Dextrose (d.-glucose), GLYCOSE, GRAPE-SUGAR, and DIABETIC SUGAR, occurs abundantly in the grape, and also, often accompanied with levulose (d.-fructose), in honey, sweet fruits, seeds, roots, etc. It occurs in the intestinal tract during digestion, also in small quantities in the blood and lymph, and as traces in other animal fluids and tissues. It only occurs as traces in urine under normal conditions, while in diabetes the quantity is very large. It is formed in the hydrolytic cleavage of starch, dextrin, and other compound carbohydrates, as also in the splitting of glucosides.

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[1] Journ. f. prakt. Chem., Bd. 33, and Ber. d. deutsch. chem. Gesell., Bdd. 20, 21, 22.

[2] *Ibid.*, Bd. 21.

[3] Biolog. Centralbl., Bd. 12, S. 321 and 481.

Properties of Dextrose. Dextrose crystallizes sometimes with 1 mol. water of crystallization in warty masses or small leaves or plates, and sometimes when free from water in needles. The sugar containing water of crystallization melts even below 100° C. and loses its water of crystallization at 110° C. The anhydrous sugar melts at 146° C., and is converted into glucosan, $C_6H_{10}O_5$, at 170° C. with the elimination of water. On strongly heating it is converted into caramel and then decomposed.

Grape-sugar is readily soluble in water. This solution, which is not as sweet as a cane-sugar solution of the same strength, is dextrogyrate and shows strong birotation. The specific rotation is somewhat dependent upon concentration of the solution, but the specific rotation of a watery solution of 1–15% anhydrous dextrose at + 20° C. may be considered as + 52°.6. Dextrose dissolves sparingly in cold, but more freely in boiling, alcohol. 100 parts alcohol of sp. gr. 0.837 dissolves 1.95 parts anhydrous glucose at + 17°.5 C. and 27.7 parts at the boiling temperature (ANTHON[1]). Glucose is insoluble in ether. If an alcoholic caustic-alkali solution is added to an alcoholic solution of glucose, an amorphous precipitate of insoluble alkali compound is formed. On warming this compound it decomposes easily with the formation of a yellow or brownish color, which is the basis of the following reaction.

MOORE'S *Test.* If a glucose solution is treated with about ¼ of its volume of caustic potash or soda and warmed, the solution becomes first yellow, then orange, yellowish brown, and lastly dark brown. It has at the same time a faint odor of caramel, and this odor is more pronounced on acidification.

Glucose forms many crystallizable combinations with NaCl, of which the easiest to obtain is $(C_6H_{12}O_6)_2.NaCl + H_2O$, which forms large colorless six-sided double pyramids or rhomboids with 13.40% NaCl.

Glucose in neutral or very faintly acid (by an organic acid) solution passes into alcoholic fermentation with beer-yeast, $C_6H_{12}O_6 = 2C_2H_5.OH + 2CO_2$. The most favorable temperature for this fermentation is 34° C. according to JODLBAUER.[2] Besides the alcohol and carbon dioxide there are formed, especially at higher temperatures, small quantities of homologous alcohols (amyl-alcohol), glycerin, and succinic acid. In the presence of acid milk or cheese the grape-sugar passes, especially in the presence of a base such as ZnO or $CaCO_3$, into lactic-acid fermentation. The lactic acid may then further pass into butyric-acid fermentation: $2C_3H_6O_3 = C_4H_8O_2 + 2CO_2 + 4H$.

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[1] Cited from Tollens' Handbuch.

[2] Hoppe-Seyler's Handbuch, 6. Auf., 1893, S. 68.

Grape-sugar reduces several metallic oxides, such as copper oxide, bismuth oxide, mercuric oxide, in alkaline solutions, and the most important reactions for sugar are based on this fact.

TROMMER'S *test* is based on the property that glucose possesses of reducing copper-hydrated oxide in alkaline solution into suboxide. Treat the glucose solution with about $\frac{1}{6}$–$\frac{1}{3}$ vol. caustic soda and then carefully add a dilute copper-sulphate solution. The copper-hydrated oxide is thereby dissolved, forming a beautiful blue solution, and the addition of copper sulphate is continued until a very small amount of hydrate remains undissolved in the liquid. This is now warmed and a yellow hydrated suboxide or red suboxide separates even below the boiling-point. If too little copper salt has been added, the test will be yellowish brown in color as in MOORE'S test; but if an excess of copper-salt has been added, the excess of hydrate is converted on boiling into a dark-brown hydrate which interferes with the test. To prevent these difficulties the so-called FEHLING'S solution may be employed. This reagent is obtained by mixing before use equal volumes of an alkaline solution of Rochelle salts and a copper-sulphate solution (see Quantitative Estimation of Sugar in the Urine in regard to concentration). This solution is not reduced or noticeably changed by boiling. The tartrate holds the excess of copper-hydrate oxide in solution, and an excess of the reagent does not interfere in the performance of the test. In the presence of sugar this solution is reduced.

BOTTGER-ALMÉN'S *test* is based on the property glucose possesses of reducing bismuth oxide in alkaline solution. The reagent best adapted for this purpose is obtained, according to NYLANDER'S[1] modification of ALMÉN'S original test, by dissolving 4 grms. Rochelle salt in 100 parts 10% caustic-soda solution and adding 2 grms. bismuth subnitrate and digesting on the water-bath until as much of the bismuth salt is dissolved as possible. If a glucose solution is treated with about $\frac{1}{10}$ vol., or with a larger quantity of the solution when large quantities of sugar are present, and boiled for a few minutes, the solution becomes first yellow, then yellowish brown, and lastly nearly black, and after a time a black deposit of bismuth (?) settles.

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[1] Zeitschr. f. physiol. Chem., Bd. 8.

On heating with PHENYLHYDRAZIN ACETATE a dextrose solution gives a precipitate consisting of fine yellow crystalline needles which are nearly insoluble in water but soluble in boiling alcohol, and which separate again on treating the alcoholic solution with water. The crystalline precipitate consists of *phenylglucosazone*. This compound melts when pure at 204–205° C.

Glucose is not precipitated by a lead-acetate solution, but is almost completely precipitated by an ammoniacal basic lead-acetate solution. On warming the precipitate becomes flesh-color or rose-red (Rubner's *reaction*[1]).

If a watery solution of grape-sugar is treated with BENZOYLCHLORIDE and an excess of caustic soda, and shaken until the odor of benzoylchloride has disappeared, a precipitate of benzoic-acid ester of glucose will be produced which is insoluble in water or alkali (Baumann[2]).

If ½–1 c.c. of a dilute watery solution of glucose is treated with a few drops of a 15% alcoholic solution of *α-naphthol*, the liquid is colored a beautiful violet on the addition of 1–2 c.c. concentrated sulphuric acid (Molisch[3]). This reaction depends on the formation of furfurol from the sugar by the action of the sulphuric acid.

DIAZOBENZOL-SULPHONIC ACID gives with a dextrose solution made alkaline with a fixed alkali a red color, after 10–15 minutes gradually changing to violet. ORTHONITROPHENYL-PROPIOLIC ACID yields indigo when boiled with a small quantity of sugar and sodium carbonate, and this is converted into indigo-white by an excess of sugar. An alkaline solution of grape-sugar is colored deep red on being warmed with a dilute solution of PICRIC ACID.

A more complete description as to the performance of these several tests will be given in detail in a subsequent chapter (on the urine).

Dextrose is prepared pure by the following simple method of Soxhlet and Tollens, being a modification of Schwaz's[4] method:

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[1] Zeitschr. f. Biologie, Bd. 20.

[2] Ber. d. deutsch. chem. Gesellsch., Bd. 19; also Kueny, Zeitschr. f. physiol. Chem., Bd. 14.

[3] Monatshefte f. Chem., Bd. 7, and Centralbl. f. d. med. Wissensch., 1887, S. 34 and 49.

[4] Tollens' Handbuch der Kohlehydrate, S. 39.

Treat 12 litres alcohol with 480 c.c. fuming hydrochloric acid and warm to 45–50° C.; gradually add 4 kilos powdered cane-sugar, and allow to cool after heating for 2 hours, when all the sugar will have dissolved and been inverted. To incite crystallization, some crystals of anhydrous dextrose are added, and after several days the crystals are sucked dry by the air-pump, washed with dilute alcohol to remove hydrochloric acid and crystallized from alcohol or methyl alcohol. According to TOLLENS it is best to dissolve the sugar in one half its weight of water on the water-bath and then add double this volume of 90–95% alcohol.

In detecting dextrose in animal fluids or extracts of tissues we may make use of the above-mentioned reduction-tests, the optical determination, the fermentation, and phenylhydrazin tests. For the quantitative estimation the reader is referred to the chapter on urine. Those liquids containing proteids must first have these removed by coagulation with heat and addition of acetic acid, or by precipitation with alcohol or metallic salts, before testing for dextrose. In regard to the difficulties of operating with blood and serous fluids we refer the student to the works of SCHENK,[1] RÖHMANN,[2] ABELES,[3] and SEEGEN.[4]

The **guloses** are stereo-isomers of dextrose and may be prepared artificially. d-gulose is obtained on the reduction of d-gulonic acid, which is derived on the reduction of glycuronic acid (see chapter on urine).

Mannoses.—*d-mannose*, also called *seminose*, is obtained with d-fructose, on the careful oxidation of d-mannite. It is also obtained on the hydrolysis of natural carbohydrates, such as salep slime, and reserve cellulose (especially from the shavings from the ivory-nut). It is dextrorotatory, readily ferments with beer-yeast, gives a hydrazon not readily soluble in water, and an osazon which is identical with that from d-glucose.

d-fructose, also called LEVULOSE, FRUIT-SUGAR, occurs, as above stated, mixed with dextrose extensively distributed in the plant kingdom and also in honey. It is formed in the hydrolytic cleavage of cane-sugar and other carbohydrates, but it is readily obtained by the hydrolytic splitting of inulin. In extraordinary cases of diabetes mellitus we find fructose in the urine. This sugar has won special dietetic importance in diabetes on account of its being readily assimilated.

Fructose crystallizes with difficulty in needles partly anhydrous and partly containing water. It is readily soluble in water, but nearly insoluble in cold absolute alcohol, though rather readily in boiling alcohol. Its watery solution is lævogyrate, but the statements in regard to the specific rotation are quite variable. Fructose ferments with yeast, and gives the same reduction tests as dextrose and also the same osazone. It gives a combination with calcium which is less soluble than the corresponding dextrose combination.

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[1] Pflüger's Archiv, Bdd. 46 and 47.

[2] Centralbl. f. Physiol., Bd. 4.

[3] Zeitschr. f. physiol. Chem., Bd. 15.

[4] Centralbl. f. Physiol., Bdd. 4 and 8.

Fructose, as above stated, is best obtained by the hydrolytic splitting of inulin, by warming with faintly acidulated water.

Sorbinose (sorbin) is obtained from the juice of the berry of the mountain ash under certain conditions. It is crystalline and is lævogyrate, and is converted into sorbit by reduction , hence it seems to be a ketose which is stereoisomeric with fructose.

Galactose (not to be mistaken for lactose or milk-sugar) is obtained on the hydrolytic cleavage of milk-sugar and by hydrolysis of other carbohydrates, especially varieties of gums and slime bodies. It is also obtained on heating cerebrin, a nitrogenized glucoside prepared from the brain, with dilute mineral acids.

It crystallizes in needles or leaves, which melt at 168° C. It is somewhat less soluble than dextrose in water. It is dextrogyrate, and shows multirotation. It ferments with yeast (although not as rapidly as dextrose); still the statements on this subject are contradictory. Galactose reduces FEHLING's solution to a less extent than dextrose, and 10 c.c. of this solution are reduced, according to SOXHLET, by 0.0511 gm. galactose in 1% solution. Its phenylosazon melts at 193° C. On oxidation it first yields galactonic acid and then mucic acid. Both l- and i-galactose have been artificially prepared.

Talose is a sugar which is artificially prepared by the reduction of talonic acid. Talonic acid is obtained from d-galactonic acid by heating it with chinolin or pyridin to 140-150° C.

Disaccharides.

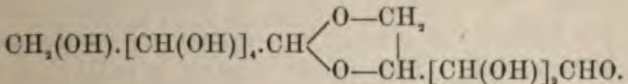
Some of the varieties of sugar belonging to this group occur ready formed in nature Thus we have cane-sugar and milk-sugar. Some, on the contrary, such as maltose and isomaltose, are produced by the partial hydrolytic cleavage of complicated carbohydrates. Isomaltose is besides this also obtained from glucose by reversion (see below).

The disaccharides or hexobioses are to be considered as anhydrides, derived from two monosaccharides with the exit of 1 mol. water. Corresponding to this, their general formula is $C_{12}H_{22}O_{11}$. On hydrolytic cleavage, on the addition of water, they yield two molecules of hexoses, and indeed either two molecules of the same hexose or two different hexoses. Thus:

$$\begin{aligned}\text{Cane-sugar} + H_2O &= \text{glucose} + \text{fructose};\\ \text{Maltose} + H_2O &= \text{glucose} + \text{glucose};\\ \text{Milk-sugar} + H_2O &= \text{glucose} + \text{galactose}.\end{aligned}$$

The fructose turns the polarized ray more to the left than the glucose does to the right; hence the mixture of hexoses obtained on the cleavage of cane-sugar has an opposite rotation to the cane-sugar itself. On this account the mixture is called INVERT SUGAR, and the hydrolytic splitting is designated as *inversion*. This term inversion is not only used for the splitting of cane-sugar, but is also used for the hydrolytic cleavage of compound sugars into monosaccharides. The reverse reaction, whereby monosaccharides are condensed into complicated carbohydrates, is called *reversion*.

We subdivide the disaccharides into two groups. One, to which cane-sugar belongs, where the members have not the property of reducing certain metallic oxides and of reacting with phenylhydrazin. The other group, on the contrary, to which the two maltoses and milk-sugar belong, the members act like monosaccharides in regard to the reduction tests, and yield osazones with phenylhydrazin. The members of this last group have the character of aldehyde-alcohols; hence they are given the following formula:



$$CH_2(OH).[CH(OH)]_4.CH\left\langle\begin{array}{l}O-CH_2\\ \qquad\;\, |\\ O-CH.[CH(OH)]_3CHO.\end{array}\right.$$

Cane-sugar or SACCHAROSE occurs extensively distributed in the plant kingdom. It occurs to greatest extent in the stalk of the sugar-millet and sugar-cane, the roots of the sugar-beet, the trunk of certain varieties of palms and maples, in carrots, etc. Cane-sugar is of extraordinarily great importance as a food and condiment.

Cane-sugar forms large, colorless monoclinic crystals. On heating it melts in the neighborhood of 160° C., and on heating stronger it turns brown, forming so-called caramel. It dissolves very readily in water, and according to SCHEIBLER[1] 100 parts saturated sugar solution contains 67 parts sugar at 20° C. It dissolves with difficulty in strong alcohol. Cane-sugar is strongly dextrorotatory. The specific rotation is only slightly modified by concentration, but is markedly changed by the presence of other inactive substances. The specific rotation is $(\alpha)D = +66°.5$.

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[1] See Tollens' Handbuch der Kohlehydrate, S. 121.

Cane-sugar acts indifferently towards MOORE's test and to the ordinary reduction tests, and it does not react with phenylhydrazin. It does not ferment directly, but ferments after inversion, which can be brought about by an enzym, invertin, contained in the yeast. An inversion of cane-sugar also takes place in the intestinal canal. Concentrated sulphuric acid blackens cane-sugar very quickly even at the ordinary temperature, and anhydrous oxalic acid acts the same on warming on the water-bath. Various products are obtained on the oxidation of cane-sugar, dependent upon the variety of oxidizing material and also upon the intensity of the action. Saccharic acid and oxalic acid are the most important products.

The reader is referred to complete text-books on chemistry for the preparation and quantitative estimation of cane-sugar.

Maltose (MALT-SUGAR) is formed in the hydrolytic cleavage of starch by malt diastase, saliva, and pancreatic juice. It is obtained from glycogen under the same conditions (see Chapter VIII). Maltose is also produced transitorily in the action of sulphuric acid on starch. Maltose forms the fermentable sugar of the potato or grain mash, and also of the beerwort.

Maltose crystallizes with 1 mol. water of crystallization in fine white needles. It is readily soluble in water, rather easily in alcohol, but insoluble in ether. Its solutions are dextrorotatory, and show birotation. The specific rotation is $(\alpha)D = +137°$. Maltose fements readily and completely with yeast, and acts like dextrose in regard to the reduction tests. It yields phenylmaltosazone on warming with phenylhydrazin for 1½ hours. This phenylmaltosazone melts at 206° C. Maltose differs from dextrose chiefly in the following: It does not dissolve as readily in alcohol, has a stronger dextrorotatory power, has a feebler reducing action on FEHLING's solution. 10 c.c. FEHLING's solution is, according to SOXHLET,[1] reduced by 77.8 milligrams anhydrous maltose in approximately 1% solution.

Isomaltose. This variety of sugar is produced, as has been shown by Fischer,[1] besides dextrin-like products, by the action of fuming hydrochloric acid on glucose. It is also formed, besides ordinary maltose, in the action of diastase on starch paste. It is also produced, with maltose, by the action of saliva or pancreatic juice (Kulz and Vogel[2]) or blood-serum (Röhmann[3]) on starch. It also occurs in beer and in technical starch-sugar.

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[1] Cit. from Tollens' Handbuch, S 152.

Isomaltose dissolves very readily in water, has a pronounced sweetish taste, ferments but slowly. It is dextrorotatory, and has very nearly the same power of rotation as maltose. Isomaltose is characterized by its osazone. This forms fine yellow needles, which begin to form drops at 140° C. and melt at 150–153° C. It is rather easily soluble in hot water.

Milk-sugar (Lactose). As this sugar occurs exclusively in the animal world, in the milk of human beings and animals, it will be treated of in a following chapter (on milk).

Trehalose is a hexobiose found in fungi. **Melebiose** is a saccharose obtained with d-fructose in the partial hydrolytic cleavage of **raffinose** (a hexotriose) occurring in beet-root molasses. Melebiose splits into galactose and glucose.

Polysaccharides.

If we exclude the hexotrioses and the few remaining sugar-like polysaccharides, this group includes a great number of very complex carbohydrates, which occur only in the amorphous condition or not as crystals in the ordinary sense. Contrary to the bodies belonging to the other groups, these have no sweet taste. Some are soluble in water, while others swell up therein, especially in warm water, and finally are neither dissolved nor visibly changed. Polysaccharides are ultimately converted into monosaccharides by hydrolytic cleavage.

The polysaccharides (not sugar-like) are ordinarily divided into the following chief groups: *starch group*, *gum* and *vegetable-mucilage group*, and *cellulose group*.

Starch Group $(C_6H_{10}O_5)x$.

Starch, Amylum. $(C_6H_{10}O_5)x$. This substance occurs in the plant kingdom very extensively distributed in the different parts of the plant, especially as reserve food in the seeds, roots, tubers, and trunk.

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[1] Ber. d. deutsch. chem. Gesellsch., Bd. 23, S. 3687.

[2] Zeitschr. f. Biologie, Bd. 31.

[3] Centralbl. f. d. med. Wissensch., 1893, S. 849.

Starch is a white, odorless, and tasteless powder, consisting of small grains, which have a stratified structure and different shape and size in different plants. According to the ordinary opinion the starch-grains consist of two different substances, STARCH GRANULOSE and STARCH CELLULOSE, of which the first only goes into solution on treatment with diastatic enzymes.

Starch is considered insoluble in cold water. The grains swell up in warm water and burst, yielding a paste. Starch is insoluble in alcohol and ether. On heating starch with water alone, or heating with glycerin to 190° C., or on treating the starch-grains with 6 parts dilute hydrochloric acid of sp. gr. 1.06 at ordinary temperature for 6 to 8 weeks,[1] it is converted into soluble starch (AMYLODEXTRIN, AMIDULIN). Soluble starch is also formed as an intermediate step in the conversion of starch into dextrose by dilute acids or diastatic enzymes. Starch-granules swell up and form a pasty mass in caustic potash or soda. This mass gives neither MOORE'S nor TROMMER'S test. Starch-paste does not ferment with yeast. The most characteristic test for starch is the blue coloration produced by iodine in the presence of hydroiodic acid or alkali iodides.[2] This blue coloration disappears on the addition of alcohol or alkalies, and also on warming, but reappears again on cooling.

On boiling with dilute acids starch is converted into glucose. In the conversion by means of diastatic enzymes we have as a rule, besides dextrin, maltose, and isomaltose, only very little glucose. We are considerably in the dark as to the kind and number of intermediate products produced in this process (see dextrin).

Starch may be detected by means of the microscope and by the iodine reaction. Starch is quantitatively estimated, according to SACHSSE'S method,[3] by converting it into sugar by hydrochloric acid and then determining the sugar by the ordinary methods.

Inulin, $(C_6H_{10}O_5)x + H_2O$, occurs in the underground parts of many compositæ, especially in the roots of the inula helenium, the tubers of the dahlia, the varieties of helianthus, etc. It is ordinarily obtained from the tubers of the dahlia.

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[1] See Tollens' Handbuch, S. 187.

[2] Mylius, Ber. d. deutsch. chem. Gesellsch., Bd. 20, S. 688, and Zeitschr. f. physiol. Chem., Bd. 11.

[3] Tollens' Handbuch, S. 184.

Inulin forms a white powder, similar to starch, consisting of sphæroid crystals, which are readily soluble in warm water without forming a paste. It separates slowly on cooling, but more rapidly on freezing. Its solutions are lævogyrate and are precipitated by alcohol, and are only colored yellow with iodine. Inulin is converted into the lævogyrate monosaccharide fructose, on boiling with dilute sulphuric acid. Diastatic enzymes have no or very slight action on inulin.[1]

Lichenin (MOSS-STARCH) occurs in many lichens, namely, in Iceland moss. It is not soluble in cold water, but swells up into a jelly. It is soluble in hot water, forming a jelly on allowing the concentrated solution to cool. It is colored yellow by iodine, and yields glucose on boiling with dilute acids. Lichenin is not changed by diastatic enzymes such as ptyalin or amylopsin (NILSON[2]).

Glycogen. This carbohydrate, which stands to a certain extent between starch and dextrin, is principally found in the animal kingdom, hence it will be treated in a subsequent chapter (on the liver).

The Gums and Vegetable Mucilages $(C_6H_{10}O_5)x$.

These bodies may be divided into two chief groups, according to their origin and occurrence, namely, the *dextrin group* and the *vegetable gums* or mucilages. The dextrines stand in close relationship to the starches and are formed therefrom as intermediate products in the action of acids and diastatic enzymes. The various kinds of vegetable gums and vegetable mucilages occur, on the contrary, as natural products in the plant kingdom, and some may be separated from certain plants as amorphous, transparent masses and others may be extracted from certain parts of the plant, such as the wood and seeds, by proper solvents.

The dextrines yield as final products only hexoses, and indeed only dextrose on complete hydrolysis. The vegetable gums and the mucilages yield, on the contrary, not only hexoses, but also an abundance of pentoses (gum arabic and wood-gum). d-galactose occurs often amongst the hexoses, and as differentiation from the dextrines they yield mucic acid on oxidation with nitric acid. The dextrines, as well as the ordinary varieties of gums and mucilages, are precipitated by alcohol. Basic lead acetate precipitates the gums and mucilages, but not the dextrins.

[1] Tollens' Handbuch, S. 203.

[2] Upsala Läkaref. forh., Bd. 28.

Dextrin (British gum) is produced on heating starch to 200–210° C., or by heating starch, which has previously been moistened with water containing a little nitric acid, to 100–110° C. Dextrins are also produced by the action of dilute acids and diastatic enzymes on starch. We are not quite clear in regard to the steps taking place in the above processes, but the ordinary views are as follows: Soluble starch is the first product, from which a dextrin, *erythrodextrin*, which is colored red by iodine, and sugar are formed by hydrolytic splitting. On further splitting of this erythrodextrin more sugar and a dextrin, *achroodextrin*, which is not colored by iodine, is formed. From this achroodextrin after successive splittings we have sugar and dextrins of lower molecular weights formed, until finally we have sugar and a dextrin, *maltodextrin*, which refuses to split further, as final products. The views are rather contradictory in regard to the number of dextrins which occur as intermediate steps. The sugar formed is isomaltose, from which maltose and very little dextrose are produced. Another view is that first several dextrins are formed consecutively in the successive splitting with hydration, and then finally the sugar is formed by the splitting of the last dextrin.[1]

The various dextrins have not as yet been separated from each other, nor isolated as chemical individuals; hence the characteristic properties and reactions can only be given for the dextrins in general.

The dextrins appear as an amorphous, white or yellowish-white powder which is readily soluble in water. Their concentrated solutions are viscid and sticky, similar to gum solutions. The dextrins are dextrogyrate, the specific rotation of maltodextrin being $(\alpha)D = +174°.5$. They are insoluble or nearly so in alcohol, and insoluble in ether. Watery solutions of dextrins are not precipitated by basic lead acetate. Dextrins dissolve copper oxyhydrate in alkaline liquids, forming a beautiful blue solution. The question whether or not perfectly pure dextrin reduces FEHLING'S solution is undecided. According to BRÜCKE[2] a non-reducible dextrin may be obtained by warming a solution of achroodextrin with an excess of alkaline copper solution and then precipitating with alcohol. According to SCHEIBLER and MITTELMEIER[1] the dextrin obtained by the action of acid is a polysaccharide of an aldehydic nature, hence it acts as a reducing agent. The dextrins are not directly fermentable. The behavior of the various dextrins to iodine has been given above, but it must be remarked that, according to MUSCULUS and MEYER,[2] erythrodextrin is only a mixture of achroodextrin with a little soluble starch.

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[1] In regard to the new theories see Lintner and Düll, Ber. d. deutsch. chem. Gesellsch., Bd. 26, S. 2533, and Scheibler and Mittelmeier, *ibid.*, Bd. 23, S. 3060, and Bd. 26. S. 2930.

[2] Vorlesungen über Physiologie. Wien, 1874. S. 231.

The **vegetable gums** are soluble in water, forming solutions which are viscid but may be filtered. We designate, on the contrary, as **vegetable mucilages** those varieties of gum which do not or only partly dissolve in water, and which swell up therein to a greater or less extent. The natural varieties of gum and mucilage to which several generally known and important substances, such as gum arabic, wood-gum, cherry-gum, salep and quince mucilage, and probably also the little-studied pectin substances, belong will not be treated of in detail, because of their unimportance from a zoophysiological standpoint.

The Cellulose Group $(C_6H_{10}O_5)x$.

Cellulose is that carbohydrate, or perhaps more correctly mixture of carbohydrates, which forms the chief constituent of the walls of the plant-cells. This is true for at least the walls of the young cells, while in the walls of the older cells the cellulose is extensively incrusted with a substance called LIGNIN.

The true celluloses are characterized by their great insolubility. They are insoluble in cold or hot water, alcohol, ether, dilute acids, and alkalies. We have only one specific solvent for cellulose, and that is an ammoniacal solution of copper oxide called SCHWEITZER's reagent. The cellulose may be precipitated from this solvent by the addition of acids, and obtained as an amorphous powder after washing with water.

Cellulose is converted into a substance, so-called AMYLOID, which gives a blue coloration with iodine by the action of concentrated sulphuric acid. By the action of strong nitric acid or a mixture of nitric acid and concentrated sulphuric-acid celluloses is converted into nitric-acid esters or nitro-cellulose, which are highly explosive and have found great practical use.

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[1] Ber. d. deutsch. chem. Gesellsch., Bd. 23, S. 3060, and Bd. 26, S. 2930.

[2] Zeitschr. f. physiol. Chem., Bd. 4, S. 451.

The ordinary celluloses when treated at the ordinary temperature with strong sulphuric acid and then boiled for some time after diluting with water is converted into dextrose. Other varieties of cellulose have a different behavior, namely, we have a cellulose which yields mannose on the preceding treatment. This substance, called mannoso-cellulose by E. SCHULZE,[1] occurs in the coffee-bean, as well as in the cocoanut and sesame cake, and is not to be considered as belonging to the hemicellulose group.

Hemicelluloses are, according to E. SCHULZE, those constituents of the cell-wall related to cellulose which differ from the ordinary cellulose by dissolving on heating with strongly diluted mineral acids, such as 1.25% sulphuric acid, with a splitting into monosaccharides. The sugars produced hereby are of different kinds. The hemicellulose from the yellow lupin yields galactose and arabinose, from the rye and wheat bran arabinose and xylose, and from the ivory-nut—called RESERVE CELLULOSE by REISS[2]—mannose.

The cellulose, at least in part, undergoes decomposition in the intestinal tract of man and animals. A closer discussion of the nutritive value of cellulose will be given in a future chapter (on digestion). The great importance of the carbohydrates in the animal economy and to animal metabolism will also be given in following chapters.

[1] Zeitschr. f. physiol. Chem., Bd. 16.

[2] Ber. d. deutsch. chem. Gesellsch., Bd. 22.

CHAPTER IV.

THE ANIMAL FATS.

THE fats form the third chief group of the organic foods of man and animals. They occur very widely distributed in the animal and plant kingdoms. Fat occurs in all organs and tissues of the animal organism, though the quantity may be so variable that a tabular exhibit of the amount of fat in different organs is of little interest. The marrow contains the largest quantity, having over 960 p. m. The three most important deposits of fat in the animal organism are the intermuscular connective tissue, the fatty tissue in the abdominal cavity, and the subcutaneous connective tissues. Amongst the plants the seeds and fruit, and in certain instances also the roots, are rich in fat.

The fats consist nearly entirely of so-called neutral fats with only very small quantities of fatty acids. The neutral fats are esters of the triatomic alcohol, glycerin, with monobasic fatty acids. These esters are triglycerides, that is, the three hydrogen atoms of the hydroxyl of the glycerin are replaced by the fatty-acid radicals, and their general formula is therefore $C_3H_5.O_3.R_3$. The animal fats consist chiefly of esters of the three fatty acids, stearic, palmitic, and oleic acids. In the plant kingdom triglycerides of other fatty acids, such as lauric acid, linoleic acid, erucic acid, etc., sometimes occur abundantly.

The animal fats are of the greatest interest and consist of a mixture of varying quantities of TRISTEARIN, TRIPALMITIN, and TRIOLEIN, having an average elementary composition of C 76.5, H 12.0, and O 11.5 per cent.

Fats from different species of animals, and even from different parts of the same animal, have an essentially different consistency, depending upon the relative amounts of the different fats. In solid fats—as tallow—tristearin and tripalmitin are in excess, while the less solid fats are characterized by a greater abundance of tripalmitin and triolein. This last-mentioned fat is found in greater quantities proportionally in cold-blooded animals, and this accounts for the fat of these animals remaining fluid at temperatures at which the fat of warm-blooded animals solidifies. Human fat from different organs and tissues contains, in round numbers, 670–800 p. m. triolein. The melting-point of different fats depends upon the composition of the mixtures, and it not only varies for fat from different tissues of the same animal, but also for the fat from the same tissues in various kinds of animals.

Neutral fats are colorless or yellowish and, when perfectly pure, odorless and tasteless. They are lighter than water, on which they float when in a molten condition. They are insoluble in water, dissolve in boiling alcohol, but separate on cooling,—often in crystals. They are easily soluble in ether, benzol, and chloroform. The fluid neutral fats give an emulsion when shaken with a solution of gum or albumin. With water alone they give an emulsion only after vigorous and prolonged shaking, but the emulsion is not persistent. The presence of some soap causes a very fine and permanent emulsion to form easily. Fat produces spots on paper which do not disappear; it is not volatile; it boils at about 300° C. with partial decomposition, and burns with a luminous and smoky flame. The fatty acids have most of the above-mentioned properties in common with the neutral fats, but differ from them in being soluble in alcohol-ether, in having an acid reaction, and by not giving the acrolein test. The neutral fats generate a strong irritating vapor of acrolein, due to the decomposition of glycerine, $C_3H_5(OH)_3 - 2H_2O = C_3H_4O$, when heated alone, or more easily when heated with potassium bisulphate or with other substances removing water.

The neutral fats may be split by the addition of the constituents of water according to the following equation: $C_3H_5(OR)_3 + 3H_2O = C_3H_5(OH)_3 + 3HOR$. This splitting may be produced by the pancreatic enzyme or by superheated steam. We most frequently decompose the neutral fats by boiling them with caustic alkali not too concentrated, or, still better (in zoochemical researches), with an alcoholic potash solution. By this procedure, which is called saponification, the alkali salts of the fatty acids (soaps) are formed. If the saponification is made with lead oxide, then lead-plaster, lead-salt of the fatty acids, is produced. We do not only call the splitting of neutral fats by alkalies saponification, but also the splitting of neutral fats into fatty acids and glycerin in general.

On keeping fats for a long time in contact with air they undergo a change, becoming yellow in color, acid in reaction, and develop an unpleasant odor and taste. It becomes *rancid*, and in this change a part of the fat is split into fatty acids and glycerin, and then an oxidation of the free fatty acids takes place, producing volatile bodies of an unpleasant odor. The rancidity is not due, as shown by GAFFKY and RITSERT,[1] to the presence of microbes. According to these investigators the change is due to the combined action of air and light.

In certain animal fats, as in milk-fat, small quantities of triglycerides of lower fatty acids, such as butyric, caproic acids, etc., occur. The same is observed in fat from certain animals, although little studied. Still these are of minor importance as compared to the three most important fats of the animal body, namely, *tristearin*, *tripalmitin*, and *triolein*.

Stearin, or TRISTEARIN, $C_3H_5(C_{18}H_{35}O_2)_3$, occurs especially in the solid varieties of tallow, but also in the vegetable fats.

Stearic acid, $C_{18}H_{36}O_2$, is found in the free state in decomposed pus, in the expectorations in gangrene of the lungs, and in cheesy tuberculous masses. It occurs as lime-soap in excrements and adipocere, and in this last product also as an ammonia soap. It perhaps exists as sodium soap in the blood, transudations, and pus.

Stearin is the hardest and most insoluble of the three ordinary neutral fats. It is nearly insoluble in cold alcohol and soluble with great difficulty in cold ether (225 parts). It separates from warm alcohol on cooling as rectangular, less frequently as rhombical plates. The statements in regard to the melting-point are somewhat varied. Pure stearin, according to HEINTZ,[2] melts between + 55° and 71°.5. The stearin from the fatty tissues (not pure) melts at + 63° C.

Stearic acid crystallizes (on cooling from boiling alcohol) in large, shining, long-rhombical scales or plates. It is less soluble than the other fatty acids and melts at 69.2° C. Its barium salt contains 19.49% barium.

Palmitin, TRIPALMITIN, $C_3H_5(C_{16}H_{31}O_2)_3$. Of the two solid varieties of fats, palmitin is the one which occurs in predominant quantities in human fat (LANGER).[1] Palmitin is present in all animal fats and in several kinds of vegetable fats. A mixture of stearin and palmitin was formerly called MARGARIN.

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[1] Naturwissenschaftl. Wochenschr., 1890.

[2] Annal. d. Chem. u. Pharm., Bd. 92, S. 300.

Palmitic acid, $C_{16}H_{32}O_2$. As to occurrence, about the same remarks apply as to stearic acid. The mixture of these two acids has been called margaric acid, and this mixture occurs—often as very long, thin, crystalline plates—in old pus, in expectorations from gangrene of the lungs, etc.

Palmitin crystallizes, on cooling from a warm saturated solution in ether or alcohol, in starry rosettes of fine needles. The mixture of palmitin and stearin, called margarin, crystallizes, on cooling from a solution, as balls or round masses which consist of short or long, thin plates or needles which often appear like blades of grass. Palmitin, like stearin, has a variable melting and solidifying point, depending upon the way it has been previously treated. The melting-point is often given as + 62°. According to other statements[2] it melts at 50°.5 C., solidifies on further heat and melts again at 66°.50 C.

Palmitic acid crystallizes from an alcoholic solution in tufts of fine needles. It melts at + 62° C.; still the admixture with stearic acid, as HEINTZ has shown, essentially changes the melting and solidifying points according to the relative amounts of the two acids. Palmitic is somewhat more soluble in cold alcohol than stearic acid; but they have about the same solubility in boiling alcohol, ether, chloroform, and benzol.

Olein, TRIOLEIN, $C_3H_5(C_{18}H_{33}O_2)_3$, is present in all animal fats and in greater quantities in plant fats. It is a solvent for stearin and palmitin. **Oleic acid,** ELAIC ACID, $C_{18}H_{34}O_2$, occurs probably as soaps in the intestinal canal during digestion and in the chyle.

Olein is, at ordinary temperatures, a nearly colorless oil of a specific gravity of 0.914, without odor or marked taste. It solidifies in crystalline needles at − 5° C. It becomes rancid quickly if exposed to the air. It dissolves with difficulty in cold alcohol, but more easily in warm alcohol or in ether. It is converted into its isomer, ELAIDIN, by nitrous acid.

Oleic acid forms at ordinary temperature a colorless, tasteless, and odorless oily liquid which solidifies in crystals at about + 4° C., which then melt again at + 14° C. On being heated it yields, besides volatile fatty acids, SEBACIC ACID, $C_{10}H_{18}O_4$, which crystallizes in shining plates and melts at + 127° C. Oleic acid is converted by nitrous acid into its isomer, ELAIDIC ACID, which is a solid, melting at + 45° C. Oleic acid is insoluble in water, but dissolves in alcohol, ether, and chloroform. With concentrated sulphuric acid and some cane-sugar it gives a beautiful red or reddish-violet liquid whose color is similar to that produced in PETTENKOFER's test for bile-acids.

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[1] Monatshefte f. Chem., Bd. 2.

[2] R. Benedikt. Analyse der Fette. Berlin, 1886. S 29.

If the watery solution of the alkali combinations of oleic acid is precipitated with lead acetate, a white, tough, sticky mass of lead oleate is obtained which is not soluble in water and only slightly in alcohol, but is soluble in ether (*differing* from the lead-salts of the other two fatty acids).

An acid related to oleic acid, DOEGLIC ACID, which is solid at 0° C., liquid at + 16°, and soluble in alcohol, is found in the blubber of the *Balæna rostrata*. KURBATOFF[1] has demonstrated the presence of linoleic acid in the fat of the silurus, sturgeon, seal, and certain other animals.

To detect the presence of fat in an animal fluid or tissue the fat must first be extracted with ether. After the evaporation of the ether the residue is tested for fat and the acrolein test must not be neglected. If this test gives positive results, then neutral fats are present; if the results are negative, then only fatty acids are present. If the above residue after evaporation gives the acrolein test, then a small portion is dissolved in alcohol-ether free from acid and which has been colored bluish violet by tincture of alkanet. If the color becomes red, a mixture of neutral fat and fatty acids is present. In this case the fat is treated in the warmth with a soda solution and evaporated on the water-bath, constantly stirring until all the water is removed. The fatty acids hereby combine with the alkali, forming soaps, while the neutral fats are not saponified under these conditions. If this mixture of soaps and neutral fats is treated with water and then shaken with pure ether, the neutral fats are dissolved, while the soaps remain in the watery solution. The fatty acids may be separated from this solution by the addition of a mineral acid which sets the acid free.

The neutral fats separated from the soaps by mean of ether are often contaminated with cholesterin, which must be separated in quantitative determinations by saponification with alcoholic caustic potash. The cholesterin is not attacked by the caustic alkali, while the neutral fats are saponified. After the evaporation of the alcohol the residue is dissolved in water and shaken with ether, which dissolves the cholesterin. The fatty acids are separated from the watery solution of the soaps by the addition of a mineral acid. If a mixture of soaps, neutral fats, and fatty acids is originally present, it is treated first with water, then agitated with ether free from alcohol, which dissolves the fat and fatty acids, while the soaps remain in the solution, with the exception of a very small amount which is dissolved by the ether.

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[1] Maly's Jahresber., Bd. 22.

To detect and to separate the different varieties of neutral fats from each other it is best first to saponify them with alcoholic potash, or still better with sodium alcoholate, according to KOSSEL, OBERMÜLLER, and KRÜGER.[1] After the evaporation of the alcohol they are dissolved in water and precipitated with sugar of lead. The lead oleate is then separated from the other two lead-salts by repeated extraction with ether. The residue insoluble in ether is decomposed on the water-bath with an excess of soda solution, evaporated to dryness, finely pulverized, and extracted with boiling alcohol. The alcoholic solution is then fractionally precipitated by barium acetate or barium chloride. In one fraction the amount of barium is determined, and in the other the melting-point of the fatty acid set free by a mineral acid. The fatty acids occurring originally in the animal tissues or fluids as free acids or as soaps are converted into barium salts and investigated as above.

The fats are poor in oxygen but rich in carbon and hydrogen. They therefore represent a large amount of chemical potential energy, and they correspondingly yield large quantities of heat on combustion. They take first rank amongst the foods in this regard and are therefore of very great importance in animal life. We will speak more in detail of this significance, also of fat formation and the behavior of the fats in the body, in the following chapters.

The LECITHINS, which stand in close relationship to the fats, will be treated of in a subsequent chapter. The following bodies append themselves to the ordinary animal fats.

Spermaceti. In the living spermaceti or white whale there is found in a large cavity in the skull an oily liquid called spermaceti, which on cooling after death separates into a solid crystalline part, ordinarily called SPERMACETI, and into a liquid, SPERMACETI-OIL. This last is separated by pressure. Spermaceti is also found in other whales and in certain species of dolphin.

The purified, solid spermaceti, which is called CETIN, is a mixture of esters of fatty acids. The chief constituent is the cetyl-palmitic ester mixed with small quantities of compound ethers of lauric, myrisitic, and stearic acids with radicals of the alcohols, LETHAL, $C_{12}H_{25}.OH$, METHAL, $C_{14}H_{29}.OH$, and STETHAL, $C_{18}H_{37}.OH$.

Cetin is a snow-white mass shining like mother-of-pearl, crystallizing in plates, brittle, fatty to the touch, and which has a varying melting-point of + 30° to 50° C., depending upon its purity. Cetin is insoluble in water, but dissolves easily in cold ether or volatile and fatty oils. It dissolves in boiling alcohol, but crystallizes on cooling. It is saponified with difficulty by a solution of caustic potash in water, but with an alcoholic solution it saponifies readily and the above-mentioned alcohols are set free.

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[1] Zeitschr. f. physiol. Chem., Bdd. 14, 15, and 16

Ethal, or cetyl alcohol, $C_{16}H_{33}.OH$, which also occurs in the coccygeal gland of ducks and geese (De Jonge[1]) and in smaller quantities in beeswax, forms white, transparent, odorless, and tasteless crystals which are insoluble in water but dissolve easily in alcohol and ether. Ethal melts at 49.5° C.

Spermaceti-oil yields on saponification valerianic acid, small amounts of solid fatty acids, and physetoleic acid. This acid forms colorless and odorless, needle-shaped crystals which easily dissolve in alcohol and ether and melt at + 34° C.

Beeswax may be treated here as concluding the subject of fats. It contains three chief constituents: 1 Cerotic acid, $C_{27}H_{54}O_2$, which occurs as cetyl ether in Chinese wax and as free acid in ordinary wax. It dissolves in boiling alcohol and separates as crystals on cooling. The cooled alcoholic extract of wax contains (2) cerolein, which is probably a mixture of several bodies, and (3) myrisin, which forms the chief constituent of that part of wax which is insoluble in warm or cold alcohol. Myrisin consists chiefly of palmitic-acid ether of melissyl (myricyl) alcohol, $C_{30}H_{61}.OH$. This alcohol is a silky, shining, crystalline body melting at + 85° C.

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[1] Zeitschr. f. physiol. Chem., Bd. 3.

CHAPTER V.

THE ANIMAL CELL.

THE *cell* is the unit of the manifold, variable forms of the organism; it forms the simplest physiological apparatus, and as such is the seat of chemical processes. It is generally admitted that all chemical processes of importance do not take place in the animal fluids, but transpire in the cells, which may be considered as the chemical laboratory of the organism. It is also principally the cells which, through their greater or less activity, regulate or govern the range of the chemical processes and also the intensity of the total exchange of material.

It is natural that the chemical investigation of the animal cell should in most cases coincide with the study of those tissues of which it forms the chief constituent. Only in a few cases can the cells be directly, by relatively simple manipulations, isolated in a rather pure state from the tissues, as, for example, in the investigation of pus or of tissue very rich in cells. But even in these cases the chemical investigation may not lead to any positive results in regard to the constituents of the uninjured living cells. By the process of chemical transformation new substances may be formed on the death of the cell, and at the same time physiological constituents of the cell may be destroyed or transported into the surrounding menstruum and therefore escape investigation. For this and other reasons we possess only a very limited knowledge of the constituents and the composition of the cell, especially of the living one.

While young cells of different origin in the early period of their existence may show a certain similarity in regard to form and chemical composition, they may, on further development, not only take the most varied forms, but may also offer from a chemical standpoint the greatest diversity. As a description of the constituents and composition of the different cells occurring in the animal organism is nearly equivalent to a demonstration of the chemical properties of most animal tissues, and as this exposition will be found in their respective chapters, we will here only discuss the chemical constituents of the young cells or the cells in general.

In the study of these constituents we are confronted with another difficulty, namely, we must differentiate by chemical research between those constituents which are essentially necessary for the life of the cells and those which are casual, i.e., stored up as reserve material or as metabolic products. In this connection we have only been able, thus far, to learn of certain substances which seem to occur in every developing cell. Such bodies, called PRIMARY by KOSSEL,[1] are, besides water and certain mineral constituents, proteids, nucleoproteids or nuclein, lecithins, glycogen (?), and cholesterin. Those bodies which do not occur in every developing cell are called SECONDARY. Amongst these we have fat, glycogen (?), pigments, etc. It must not be forgotten that it is still possible that other primary cell constituents may exist, but unknown to us, and we also do not known whether all the primary constituents of the cell are necessary or essential for the life and functions of the same. We do not know, for example, whether the ever-present cholesterin is an excretory product of the metabolism within the cell or whether it is necessary for the life and development of the same.

Another important question is the division of the various cell constituents between the two morphological components of the cell, namely, the protoplasm and the nucleus. This is very difficult to decide for many of the constituents, nevertheless it is appropriate to differentiate between the protoplasm and the nucleus.

The Protoplasm of the developing cell consists during life of a semi-solid mass, contractile under certain conditions and readily changeable, which is rich in water and whose chief portion consists of protein substances. If the cell be deprived of the physiological conditions of life, or if exposed to destructive exterior influences, such as the action of high temperatures, of chemical agents, or indeed of distilled water, the protoplasm dies. The albuminous bodies which it contains coagulate at least partially, and other chemical changes are found to take place. The alkaline reaction of the living cell may be converted into an acid by the appearance of paralactic acid, and the carbohydrate, glycogen, which habitually occurs in the young generative cell may after its death be quickly changed and consumed.

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[1] Verhandl. der physiol. Gesellsch. zu Berlin, 1890–91, Nos. 5 and 6.

The question as to the structure of the protoplasm has been answered in various ways. According to the ordinary view the body of the cell, the CYTOPLASM, contains a network, the SPONGIOPLASM, in the meshes of which is a more homogeneous, structureless substance, HYALOPLASM. It has also been admitted that the spongioplasm consists of a special substance, *plastin*, which will be described later, and that the hyaloplasm consists chiefly of proteid. Besides this the protoplasm contains granules of various kinds which behave differently with dyes and sometimes vacuoles containing fluid.

The proteids of the protoplasm consist, according to the general view, chiefly of *globulins*. *Albumins* have also been found besides the globulins. There is no doubt at present that the albumins occur in the cells only as traces, or at least only in trifling quantities. The presence of globulins can hardly be disputed, although certain cell constituents described as globulins have been shown on closer investigation to be nucleoalbumins or nucleoproteids. This is true for the so-called β-globulin isolated from the lymphatic glands by HALLIBURTON. On the contrary, according to this investigator, the so-called α-cell globulin, coagulating at 47–50° C., and occurring in all cells, is a true globulin.[1]

In opposition to the view that the chief mass of the animal cell consists of true proteids, the author[2] expressed the opinion several years ago, that the chief mass of the protein substances of the cells does not consist of proteids in the ordinary sense, but consists of more complex phosphorized bodies, and that the globulins and albumins are to be considered as nutritive material for the cells or as destructive products in the chemical transformation of the protoplasm. This view has received substantial support by investigations within the last few years. ALEX. SCHMIDT[3] has come to the view, by investigations on various kinds of cells, that they contain only very little proteid, and that the chief mass consists of very complex protein substances. LILIENFELD has also found on a quantitative analysis of leucocytes from the thymus gland only 1.76% proteid (in the dried substance), in the ordinary sense.

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[1] See Halliburton. On the Chemical Physiology of the Animal Cell, 1893, No. 1, King's College Physiol. Laboratory.

[2] Pflüger's Arch., Bd. 36, S. 449.

[3] Alex. Schmidt, Zur Blutlehre, Leipzig. 1892.

The protein substances of the cells consist chiefly of *compound proteids*, and these are divided between the glycoproteid and the nucleo-proteid groups. It is impossible at present to state the extent of nucleoalbumins in the cells because thus far in most cases no exact difference has been made between them and the nucleoproteids. HOPPE-SEYLER[1] calls *vitellin* a regular constituent of all protoplasm. This body used to be considered as a globulin, but later researches have shown that the so-called vitelline bodies may be of various kinds. Certain vitellins seem to be nucleoalbumins, and it is therefore very probable that cells habitually contain *nucleoalbumins*.

The *nucleoproteids* take a very prominent place among the compound proteids of the cell. The various substances isolated by different investigators from animal cells, such as *tissue fibrinogen* (WOOLDRIDGE[2]), *cytoglobin* and *preglobulin* (ALEX. SCHMIDT[3]), or *nucleohiston* (KOSSEL and LILIENFELD[4]), belong to this group. The cell constituent which swells up to a sticky mass with common salt solution and called ROVIDA'S *hyaline substance*, also belongs to this group.

The above-mentioned different protein substances have only been simply designated as constituents of the cells. The next question is which of these belong to the protoplasm and which to the nucleus. At present we can give no positive answer to this question. According to KOSSEL and LILIENFELD,[5] the cell nucleus of the leucocytes contains a nucleoproteid, besides nucleins, as chief constituent, and sometimes perhaps also nucleic acid (see below), while the body of the cells contains chiefly pure proteids besides other substances, and only a little nucleoalbumin, containing a very small quantity of phosphorus. This view coincides well with the observations of LILIENFELD on the behavior of the protoplasm and cell nucleus on one side as compared with the proteids and nuclein substances with certain coloring matters; but it seems to be inconsistent with the quantitative composition of the leucocytes as found by LILIENFELD. If we admit, according to KOSSEL and LILIENFELD, that the nucleoproteid, called by them *nucleohiston*, belongs only to the nucleus of the leucocytes of the thymus gland, then 77.45 parts of the 79.21 parts of proteins in 100 parts of the dried substance belongs to the nucleus and only 1.76 parts to the protoplasm. As the lymphocytes of the thymus gland of the calf contain only one nucleus, in which the mass of the nucleus surpasses that of the cytoplasm, it is natural that the relative proportion of the various protein substances in these cells cannot be taken as a standard for the composition of other cells richer in cytoplasm.

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[1] Physiol. Chem., 1877-1881, S. 76.

[2] Die Gerinnung des Blutes. Leipzig, 1891.

[3] Zur Blutlehre.

[4] Lilienfeld, Zeitschr. f. physiol. Chem., Bd. 18.

[5] Ueber die Wahlverwandschaft der Zellelemente zu gewissen Farbstoffen. Verhandl. d. physiol. Gesellsch. zu Berlin, No. 11, 1893.

Complete investigations in regard to the distribution of protein substances in the protoplasm and nucleus of other cells have not been made. If we consider for the present that the cells rich in protoplasm contain, as a rule, only very little true proteid, we are hardly wrong in considering it probable that the protoplasm contains chiefly nucleoalbumins and compound proteids besides traces of albumin and a little globulin. These compound proteids are in certain cases glycoproteids, but otherwise nucleoproteids which differ from the nucleoproteids of the nucleus in being poorer in phosphorus, besides containing a great deal of proteid and only less of the prostetic group, and hence have no specially pronounced acid character.

The nucleoproteids of the nucleus are on the contrary, as shown by LILIENFELD and KOSSEL, rich in phosphorus and of a strongly acid character. These nucleoproteids will be treated of in speaking of the nucleins of the nucleus.

In cases in which the protoplasm is surrounded by an outer, condensed layer or a cell membrane, this envelope seems to consist of albumoid substances. In a few cases these substances seem to be closely related to elastin; in other cases, on the contrary, they seem rather to belong to the keratin group. The chemical processes by which these albumoid substances are formed from the albuminous bodies or compound proteids of the protoplasm are unknown.

Among the non-proteid substances of the cell we must first mention lecithin, which exists as a positive constituent of the protoplasm. It is difficult to say whether it also exists in the nucleus.

Lecithin. This body is, according to the investigations of STRECKER,[1] HUNDESHAGEN,[2] and GILSON,[3] an ether-like combination of glycerophosphoric acid substituted by two fatty acid radicals, with a base, cholin. Therefore there may be different lecithins according to the fatty acid contained in the lecithin molecule. One of these—*distearyllecithin*—has been closely studied by HOPPE-SEYLER and DIACONOW:[4]

$$C_{44}H_{90}NPO_9 = HO.(CH_3)_3N.C_2H_4.O(OH)PO.O.C^3H_5 : (C_{18}H_{35}O_2)_2.$$

In agreement with this, if lecithin be boiled with baryta-water it yields fatty acids, glycerophosphoric acid, and cholin. It is only slowly decomposed by dilute acids. Besides small quantities of glycerophosphoric acid (perhaps also distearylglycerophosphoric acid) we have large quantities of free phosphoric acid split off.

GLYCEROPHOSPHORIC ACID $(HO)_2PO.O.C_3H_5(OH)_2$ is a bibasic acid, which probably only occurs in the animal fluids and tissues as splitting product of lecithin. The CHOLIN, which is identical with the bases SINKALIN (in mustard-seed) and AMANITIN (in agaricus muscarius), has the formula $HO.N(CH_3)_3.C_2H_4.OH$, and is therefore considered as trimethylethoxylium hydrate. Cholin, on the contrary, is not identical with the base, NEURIN, prepared by LIEBREICH as a decomposition product from the brain, which is considered as trimethylvinylium hydrate, $HO.N(CH_3)_3.C_2H_3$. The combination of cholin with hydrochloric acid gives with platinum chloride a crystalline double combination which is easily soluble in water, insoluble in alcohol and ether, and which crystallizes in six-sided orange-colored plates. This combination is used in detecting this base.

Lecithin occurs, as HOPPE-SEYLER[5] has especially shown, widely diffused in the vegetable and animal kingdoms. According to this investigator, it occurs also in many cases in loose combination with other bodies, such as albuminous bodies, hæmoglobin, and others. Lecithin, according to HOPPE-SEYLER, is found in nearly all animal and vegetable cells thus far studied, and also in nearly all animal fluids. It is specially abundant in the brain, nerves, fish-eggs, yolk of the egg, electrical organs of the *Torpedo electricus*, semen and pus, and also in the muscles and blood-corpuscles, blood-plasma, lymph, milk, and bile, as well as in other animal juices and liquids. Lecithin is also found in pathological tissues or liquids.

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[1] Annal. d. Chem. u. Pharm., Bd. 148.

[2] Journ. f. prakt. Chem., Bd. 28.

[3] Zeitschr. f. physiol. Chem., Bd. 12.

[4] Hoppe-Seyler's Med. chem. Untersuch., S. 221 and 405.

[5] Physiol. Chem., 1877–1881, p. 57.

Lecithin may be obtained in grains or warty masses composed of small crystalline plates by strongly cooling its solution in strong alcohol. In the dry state it has a waxy appearance, is plastic and soluble in alcohol, especially on heating (to 40–50° C.); it is less soluble in ether. It is dissolved also by chloroform, carbon disulphide, benzol, and fatty oils. It swells in water to a pasty mass which shows under the microscope slimy, oily drops and threads, so-called myelin forms (see Chapter XII). On warming this swollen mass or the concentrated alcoholic solution, decomposition takes place with the production of a brown color. On allowing the solution or the swollen mass to stand, decomposition takes place and the reaction becomes acid. In putrefaction lecithin yields glycerophosphoric acid and cholin; the latter further decomposes with the formation of methylamin, ammonia, carbon dioxide, and marsh-gas (HASEBROEK[1]). If dry lecithin be heated it decomposes, takes fire and burns, leaving a phosphorized coke. On fusing with caustic alkali and saltpetre it yields alkali phosphates. Lecithin is easily carried down during the precipitation of other compounds such as the proteid bodies, and may therefore very greatly change the solubilities of the latter.

Lecithin combines with acids and bases. The combination with hydrochloric acid gives with platinum chloride a double salt which is insoluble in alcohol, soluble in ether, and which contains 10.2% platinum.

It may be prepared tolerably pure from the yolk of the hen's egg by the following methods, as suggested by HOPPE-SEYLER and DIACONOW.[2] The yolk, deprived of albumin, is extracted with cold ether until all the yellow color is removed. Then the residue is extracted with alcohol at 50–60° C. After the evaporation of the alcoholic extract at 50–60° C., the sirupy matter is treated with ether and the insoluble residue dissolved in as little alcohol as possible. On cooling this filtered alcoholic solution to − 5° to − 10° C. the lecithin gradually separates in small granules. The ether, however, contains considerable of the lecithin. The ether is distilled off and the residue dissolved in chloroform and the lecithin precipitated from this solution by means of aceton (ALTMANN[1]).

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[1] Zeitschr. f. physiol. Chem., Bd. 12.

[2] Hoppe-Seyler's Med.-chem. Untersuch.

According to GILSON, a new portion of lecithin may be obtained from the ether used in extracting the yolk by dissolving the residue after the evaporation of the ether in petroleum ether and then shaking this solution with alcohol. The petroleum ether takes the fat, while the lecithin remains dissolved in the alcohol and may be obtained therefrom rather easily by using the proper precautions.

The detection and the quantitative estimation of lecithin in animal fluids or tissues is based on the solubility of the lecithin (at 50–60° C.) in alcohol-ether, by which the phosphoric acid or glycerophosphoric acid salts which may be present at the same time are not dissolved. The alcohol-ether extract is evaporated, the residue dried and fused with soda and saltpetre. Phosphoric acid is formed from the lecithin, and it can be used in the detection and quantitative estimation. The distearyllecithin yields 8.798% P_2O_5. This method is, however, not exactly correct, for it is possible that other phosphorized organic combinations, such as jecorin (see Chapter VIII) and protagon (Chapter XII) may have passed into the alcohol-ether extract. The residue of the evaporated alcohol-ether extract may be boiled for an hour with baryta-water, filtered, the excess of barium precipitated with CO_2, and filtered while hot. The filtrate is concentrated to a sirupy consistency, extracted with absolute alcohol, and the filtrate precipitated with an alcoholic solution of platinum chloride. The precipitate after filtration may be dissolved in water and allowed to crystallize over sulphuric acid.

Protagons, which are found in the leucocytes and pus cells, are also to be considered as a constituent of protoplasm. These phosphorized bodies occur principally in the brain and nerves and hence will be described in a following chapter.

Glycogen, discovered by CL. BERNARD and HENSIN, is found in developing animal cells and especially in developed embryonic tissues. According to HOPPE-SEYLER it seems to be a never-failing constituent of the cells, which show amœboidal movement, and he found this carbohydrate in the leucocytes, but not in the developed motionless pus-corpuscles.

SALOMON and afterwards others have, however, found glycogen in pus.[2] From the relationship which seems to exist between glycogen and muscular work (see Chapter XI), it is presumable that a consumption of glycogen takes place in the movement of animal protoplasm. On the other hand, the extensive occurrence of glycogen in embryonic tissues, as also its occurrence in pathological tumors and in abundant cell-formation, speaks for the importance of this body in the formation and development of the cell.

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[1] Cited from Hoppe-Seyler's Handbuch, etc., 6. Aufl., S. 84.

[2] In regard to the literature on glycogen see Chap. VIII.

In adult animals glycogen occurs in the muscles and certain other organs, but principally in the liver; therefore it will be completely described in connection with this organ (Chapter VIII). Glycogen has been directly detected as a constituent of the protoplasm of various cells.

Another body, or perhaps more correctly a group of bodies which occur widely distributed in the animal and vegetable kingdoms, and which occur regularly in the cells, are the cholesterins. The best-known representative of this group is ordinary *cholesterin*,[1] which is the chief constituent of certain biliary calculi and exists in abundant quantities in the brain and nerves. It is hardly admissible that this body is of direct importance for the life and development of the cell. It must be considered that the cholesterin, as accepted by HOPPE-SEYLER,[2] is a cleavage product appearing in the cell during the processes of life. According to HOPPE-SEYLER the same is true for the fats, which do not occur constantly in the cells and have nothing to do in the ordinary processes of life. There is no doubt that cholesterin exists as a constituent of the protoplasm, but its existence in the nucleus is questionable.

The **cell nucleus** has a rather complex structure. It consists in part of a *mitoplasm*, which consists of fibriles which form a network, and another part, which is less solid and homogeneous, called the *hyaloplasm*. The mitoplasm differs from the hyaloplasm in a stronger affinity for many dyes. On account of this behavior the first is called the chromatic substance or *chromatin*, and the other the achromatic substance or *achromatin*.

The hyaloplasm of the nucleus is considered as a mixture of proteids. The mitoplasm seems to contain the more specific constituent of the nucleus, namely, the nuclein substances. Besides this it is alleged to also contain another substance, *plastin*. This last is less soluble than the nuclein substances and does not have the property, like them, of fixing dyes.

The chief constituents of the cell nucleus are the *nucleins*, the *nucleoproteids*, and in a few cases *nucleic acid*.

Nucleins. By the name nuclein HOPPE-SEYLER and MIESCHER[3] designated the chief constituent of the nucleus of the pus cell first isolated by them. Since it has been shown by repeated research that similar bodies occur extensively in the animal and plant kingdoms, especially in organs rich in cells, we have for some time designated as nucleins a number of phosphorized bodies which are in part derived as cleavage products from the nucleo-albumins and in part form the chief constituent of the cell nucleus.

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[1] See Chap. VIII.

[2] Physiol. Chem., S. 81.

[3] Hoppe-Seyler, Med.-chem. Untersuch., S. 452.

According to HOPPE-SEYLER, these bodies may be divided into three groups. The first, to which belongs the nuclein of yeast, pus, nucleated red blood-corpuscles, and probably of the cell nucleus in general, yield as splitting products, on boiling with acids, proteid bodies, xanthin bases, and phosphoric acid. To the second group, which yield as splitting products proteid and phosphoric acid, belongs the nuclein of the yolk of the egg and casein—in other words, the nucleo-albumins in general; and to the third group, which gives as splitting products only phosphoric acid and xanthin bases, belongs only the nuclein of the sperm of the salmon.

Those nuclein substances which do not yield nuclein bases on splitting—such, for instance, as nuclein from casein and vitellin—are to be separated from the others. KOSSEL[1] has suggested the name *paranuclein* for these nuclein substances. As the paranucleins amongst themselves are very different and have only an apparent similarity to the true nucleins, the author[2] has proposed the name *pseudonucleins* for them.

The nuclein of spermatozoa, which does not yield any proteid on splitting, shows a great similarity to the substance obtained by ALTMANN from the nucleins of HOPPE-SEYLER's first group by the action of alkalies. This substance was called *nucleic acid* by ALTMANN[3] and KOSSEL,[4] and hence this nuclein will be called nucleic acid in the future.

The nuclein of the first group is, according to KOSSEL, true nuclein or simply *nuclein*. This nuclein, which gives phosphoric acid as well as proteid and xanthin bases on splitting with acids, is considered by KOSSEL as a combination between proteid and nucleic acid.

Pseudonucleins or PARANUCLEINS. These bodies are obtained as an insoluble residue on the digestion of nucleoalbumins or phosphoglycoproteids with pepsin hydrochloric acid. Attention is called to the fact that the pseudonuclein may be dissolved by the presence of too much acid or by a too energetic peptic digestion. Pseudonucleins contain phosphorus, which, as shown by LIEBERMANN,[1] is split off as metaphosphoric acid by mineral acids. The pseudonucleins are very dissimilar. One group of these, whose most important representative is the long-known pseudonuclein from casein, yields no reducing substance on boiling with mineral acids, while the other group, to which the pseudonuclein from ichthulin belongs, does yield such a substance.

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[1] Du Bois-Reymond's Arch., 1891.

[2] Zeitschr. f. physiol. Chem., Bd. 19.

[3] Du Bois-Reymond's Arch., 1889.

[4] *Ibid.*, 1891.

The pseudonucleins are amorphous bodies insoluble in water, alcohol, and ether, but readily soluble in dilute alkalies. They are not soluble in very dilute acids, and may be precipitated from their solution in dilute alkalies by adding acid. They give the proteid reactions very strongly.

In preparing a pseudonuclein, dissolve the mother-substance in hydrochloric acid of 1–2 p. m., filter if necessary, and add pepsin solution, and allow to stand at the bodily temperature for about 24 hours. The precipitate is filtered off, washed with water, and purified by alternately dissolving in very faintly alkaline water and reprecipitating with acid.

Nucleins or TRUE NUCLEINS. These bodies are obtained as an insoluble or difficultly soluble residue on the digestion of nucleoproteids with pepsin hydrochloric acid. They are rich in phosphorus, about 5% and above, and according to LIEBERMANN[2] metaphosphoric may also be split off from the true nucleins (yeast nuclein). The nucleins are decomposed into proteid and nucleic acid by caustic alkali, and as different nucleic acids exist, so there also exist different nucleins. Certain nucleins, such as yeast nuclein and that isolated by the author[3] from the pancreas and mammary gland, give a reducing carbohydrate on boiling with dilute acids, while other nucleins, like that from the thymus gland, does not. All nucleins yield *xanthin bases* or *nuclein bases*, so called by KOSSEL, on boiling with dilute acids. The nucleins contain iron to a considerable extent. They act like rather strong acids.

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[1] Ber. d. deutsch. chem. Gesellsch., Bd. 21, and Centralbl. f. d. med. Wissensch., Bd. 27.

[2] Pflüger's Arch., Bd. 47.

[3] Zeitschr. f. physiol Chem., Bd. 19.

The nucleins are colorless, amorphous, insoluble, or only slightly soluble in water. They are insoluble in alcohol and ether. They are more or less readily dissolved by dilute alkalies. Pepsin hydrochloric acid or dilute mineral acids do not dissolve them, or only to a slight extent. The nucleins give the biuret test and MILLON'S reaction. They show a great affinity for many dyes, especially the basic ones, and take these up with avidity from watery or alcoholic solutions. On burning they yield an acid coke containing metaphosphoric acid and which is very difficult to consume. On fusion with saltpetre and soda the nucleins yield alkali phosphates. According to LIEBERMANN[1] the nucleins are combinations of proteids with metaphosphoric mixed with xanthin bases.

To prepare nucleins from cells or tissues, first remove the chief mass of proteids by artificial digestion with pepsin hydrochloric acid, lixiviate the residue with very dilute ammonia, filter, and precipitate with hydrochloric acid. The precipitate is further digested with gastric juice, washed and purified by alternately dissolving in very faintly alkaline water, and reprecipitating with an acid, washing with water, and treating with alcohol-ether. A nuclein may be prepared more simply by the digestion of a nucleoproteid. In the detection of nucleins we make use of the above-described method and testing for phosphorus in the product after fusing with saltpetre and soda. Naturally the phosphates, lecithins (and jecorin) must first be removed by treatment with acid, alcohol, and ether, respectively. We must specially call attention to the fact, as shown by LIEBERMANN,[2] of the very great difficulty in removing lecithin by means of alcohol-ether. No exact methods are known for the quantitative estimation of nucleins in organs or tissues.

Nucleic Acids. KOSSEL differentiates between the various nucleic acids by the decomposition products. All yield nuclein bases as cleavage products, but the nucleic acid from bull spermatozoa yields chiefly xanthin, while that from the calf's thymus yields only adenin. According to KOSSEL[3] it is probable that there exist four nucleic acids, one for each nuclein base, namely, an adenylic, a guanylic acid, etc. The nucleic acids thus far investigated, with the exception of *adenylic acid* from the calf's thymus, were only mixtures of several nucleic acids. Another circumstance which makes the acceptance of many nucleic acids necessary is that certain of the nucleic acids, such as those from yeast, pancreas, and the mammary glands, give reducing carbohydrates or carbohydrate groups, while the others, such as the nucleic acid from the calf's thymus, salmon, and carp sperm, do not. In the far-reaching splitting of adenylic acid with sulphuric acid KOSSEL and NEUMANN[1] obtained levulinic acid.

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[1] Centralbl. f. d. med. Wissensch., Bd. 27.

[2] Pflüger's Arch., Bd. 54.

[3] Ber. d. deutsch. chem. Gesellsch., Bd. 26, S. 2753.

A general formula for the nucleic acids cannot be given, and the composition of the different nucleic acids analyzed is naturally very different. The nucleic acids do not contain any sulphur, but do contain nitrogen and phosphorus in the relation of 3 : 1, according to KOSSEL.[2] The quantity of phosphorus is large. In the nucleic acid, with the formula $C_{29}H_{49}N_9P_3O_{22}$, obtained by MIESCHER[3] from salmon sperm, the quantity of phosphorus was over 9%.

KOSSEL[4] assumes that the nucleic acid contains a nucleus which consists of phosphorus atoms combined similar to polymetaphosphoric acids. According to LIEBERMANN[5] the nucleic acids contain metaphosphoric acid, probably the mono-acid, and he has also, as above stated, split off metaphosphoric acid from nuclein. Other acids rich in phosphorus are formed by the action of alkali or boiling water on nucleic acids. From adenylic acid and later from other nucleic acids KOSSEL and NEUMANN[6] have prepared an acid called by them *thyminic acid*, which on boiling with sulphuric acid yields a crystalline substance, *thymin*, having the formula $C_5H_6N_2O_2$.[7] From the thymin they obtained a new cleavage product, a base called *cytosin*, with the probable formula $C_{21}H_{30}N_{16}O_4 + 5H_2O$.

The nucleic acids are amorphous, white, and of a strongly acid reaction. They are readily soluble in ammoniacal or alkaline water. They are not precipitated from these solutions by an excess of acetic acid, but are precipitated by a slight excess of hydrochloric acid, especially in the presence of alcohol. They are insoluble in alcohol and ether. The nucleic acids give precipitates with proteids which have been considered as nucleins. The question whether these precipitates are real nucleins has not been settled.

Nucleic acid may be best prepared, according to ALTMANN,[8] from yeast. Each 1000 c.c. of yeast is treated with 3250 c.c. dilute caustic soda of about 3% for five minutes at the temperature of the room. The chief portion of the sodium hydrate is then neutralized with hydrochloric acid, and then acetic acid added in excess. The liquid separated from the precipitated proteids is acidified with hydrochloric acid until it contains 3–5 p. m. HCl, and then mixed with an equal volume of alcohol of the same acidity. Impure nucleic acid separates out and may be purified by dissolving in ammoniacal water and repeatedly treating, as above, with acetic acid, hydrochloric acid, and alcohol.

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[1] Sitzungsber. d. Berl. Akad. d. Wissensch., Bd. 18, 1894.

[2] Du Bois-Reymond's Arch., 1892.

[3] L. c.

[4] L. c.; see also Centralbl. f. d. med. Wissensch., 1893, S. 497.

[5] Pflüger's Arch., Bd. 47, and Centralbl. f. d. med. Wissensch., 1893, S. 465 and 737.

[6] Ber. d. deutsch. chem. Gesellsch., Bd. 26, and Sitzungsber. der Berl. Akad., l. c.

[7] Du Bois-Reymond's Arch., 1894, Physiol. Abth.

[8] *Ibid.*, 1889, Physiol. Abth., S. 524.

Plastin.—On the solution of the nucleins from cell nuclei of certain plants in dilute soda solution a residue is obtained which is characterized by its great insolubility. This substance, of which the spongioplasm of the body of the cell and the nucleus granules are alleged to be composed, is considered as a nuclein modification of great insolubility, although its nature is not known.

Nucleoproteids with relatively high percentage of phosphorus and of a markedly acid character occur in cell nuclei. Like the nucleins they are also combinations of proteid with nucleic acid. They are, however, richer in proteid than the nucleins, and differ from them in that their neutral solutions decompose with the splitting off of coagulated proteid on boiling, and also in that they yield nucleins on their peptic digestion. Among the nucleoproteids the most carefully studied is nucleohiston.

Nucleohiston is the name given by KOSSEL and LILIENFELD[1] to the nucleoproteid isolated by them from the calf's thymus. Its composition is: C 48.46; H 7.00; N 16.86; P. 3.025; S 0.701; O 23.95%. On heating its solution it splits into coagulated proteid. On peptic digestion it yields nuclein. On treating with hydrochloric acid of 0.8% it splits into nuclein and a proteid substance soluble in hydrochloric acid, and which differs from other proteids in being insoluble in an excess of ammonia. KOSSEL has called this substance *histon.*

Nucleohiston is precipitated from a neutral solution by means of acetic acid, and is not redissolved by an excess of acetic acid. The neutral solution is precipitated by alcohol, but not on saturating with $MgSO_4$. Nucleohiston is easily dissolved in dilute alkalies or alkali carbonates. It is soluble in glacial acetic acid, hydrochloric and sulphuric acids. The relationship of the nucleins and histon to the coagulation of the blood will be spoken of in Chapter VI.

Nucleohiston is prepared by precipitating the filtered watery extract of the gland, free from cellular elements, with acetic acid, and purifying by repeated solution in water slightly alkaline with soda and precipitating with acetic acid. Finally it is washed with water containing acetic acid and then with alcohol, then extracted with cold and hot absolute alcohol and lastly with ether.

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[1] Zeitschr. f. physiol. Chem., Bd. 18.

The compound proteids[1] described by other investigators under the names *tissue fibrinogen* and *cell fibrinogen* are to be considered as impure nucleohiston or bodies very closely related thereto. The *cytoglobin* and *preglobulin* described by ALEX. SCHMIDT[2] as important cell constituents also belong to the same group as the nucleohiston. Cytoglobin is to be considered as the alkali combination of preglobulin. The residue remaining on the complete exhaustion of the cells with alcohol, water, and common-salt solution is called *cytin* by ALEX. SCHMIDT. The relationship of these bodies to the coagulation of blood will be spoken of in Chapter VI.

Among the decomposition products of nuclein substances the xanthin bases are of especially great interest.

Xanthin Bases. With this name we designate a group of bodies consisting of *carbon*, *hydrogen*, *nitrogen*, and in most cases also of *oxygen*, which, by their composition, show a relationship not only among themselves, but also with uric acid. These bodies are *xanthin*, *hypoxanthin*, *episarkin*, *guanin*, *adenin*, *heteroxanthin*, *paraxanthin*, and *carnin*. The bodies THEOBROMIN and THEOPHYLLIN (both dimethylxanthin) and CAFFEIN (trimethylxanthin) occurring in the vegetable kingdom also belong to this group.

The composition of these bodies occurring in the animal body is as follows:

Uric acid	$C_5H_4N_4O_3$
Xanthin	$C_5H_4N_4O_2$
Heteroxanthin (methylxanthin)	$C_6H_6N_4O_2$
Paraxanthin (dimethylxanthin)	$C_7H_8N_4O_2$
Guanin	$C_5H_5N_5O$
Hypoxanthin	$C_5H_4N_4O$
Adenin	$C_5H_5N_5$
Episarkin	$C_4H_6N_3O$ (?)
Carnin	$C_7H_8N_4O_3$

After SALOMON[3] had shown the occurrence of xanthin bases in young cells the importance of the xanthin bases as decomposition products of cell nuclei and of nucleins was shown by the pioneering researches of KOSSEL, who discovered adenin and theophyllin. In those tissues in which, as in the glands, the cells have kept their original state the xanthin bases are not found free, but in combination with other atomic groups (nucleins). In such tissue, on the contrary, as in muscles, which are poor in cell nuclei, the xanthin bases are found in the free state. As the xanthin bases, as suggested by KOSSEL, stand in close relationship to the cell nucleus, it is easy to understand why the quantity of these bodies is so greatly increased when large quantities of nucleated cells appear in such places as were before relatively poorly endowed. As an example of this we have in leucæmia blood extremely rich in leucocytes. In such blood KOSSEL[1] found 1.04 p. m. xanthin bases, against only traces in the normal blood. That the xanthin bases are also intermediate steps in the formation of urea or uric acid in the animal organism, is probable, and will be shown later (see Chapter XV).

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[1] See p. 91.

[2] Zur Blutlehre.

[3] Sitzungsber. d. Bot. Vereins der Provinz Brandenburg, 1880.

Only a few of the xanthin bases have been found in the urine or in the muscles. Only four xanthin bases—xanthin, guanin, hypoxanthin, and adenin,—have been obtained, thus far, as cleavage products of nucleins. In regard to the other xanthin bases we refer the reader to their respective chapters. Only the above four bodies, the real nuclein bases, will be treated of at this time.

Of these four bodies the xanthin and guanin form one special group, and hypoxanthin and adenin another. By the action of nitrous acid guanin is converted into xanthin and adenin into hypoxanthin.

$$\underset{\text{Guanin.}}{C_5H_4N_4O.NH} + HNO_2 = \underset{\text{Xanthin.}}{C_5H_4N_4O_2} + N_2 + H_2O;$$
$$\underset{\text{Adenin.}}{C_5H_4N_4.NH} + HNO_2 = \underset{\text{Hypoxanthin.}}{C_5H_4N_4O} + N_2 + H_2O.$$

By putrefaction guanin is converted into xanthin and adenin into hypoxanthin. On cleavage with hydrochloric acid all four of the bodies are converted into ammonia, glycocoll, carbon dioxide, and formic acid. Uric acid yields, under the same conditions, ammonia and carbon dioxide, and also glycocoll. On oxidation with hydrochloric acid and potassium chlorate xanthin, bromadenin, and bromhypoxanthin yield alloxan and urea; guanin yields guanidin, parabanic acid (an oxidation product of alloxan), and carbon dioxide. Uric acid in acid solution is oxidized into urea, alloxan and then further into parabanic acid. The close relationship of these bases to each other and to uric acid is apparent. Xanthin has been prepared synthetically by GAUTIER[2] by heating hydrocyanic acid with water and acetic acid.

The nuclein bases form crystalline salts with mineral acids, which are decomposed by water with the exception of the adenin salts. They are easily dissolved by alkalies, while with ammonia their action is somewhat different. They are all precipitated from acid solution by phosphotungstic acid, also they separate as a silver combination on the addition of ammonia and ammoniacal silver-nitrate solution. These precipitates are soluble in boiling nitric acid of 1.1 sp. gr. All xanthin bases with the exception of caffein and theobromin are precipitated by FEHLING's solution (see Chap. XV) in the presence of a reducing substance such as hydroxylamin (DRECHSEL and BALKE[1]). Copper sulphate and sodium bisulphite may also be used to advantage in their precipitation (KRÜGER[2]). This behavior of the xanthin bases is made use of in their precipitation and preparation.

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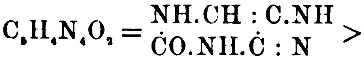
[1] Zeitschr. f. physiol. Chem., Bd. 7, S. 22.

[2] Compt. rend., Tome 98, p. 1523.



Xanthin, $C_5H_4N_4O_2 = \begin{matrix}\mathrm{NH.CH : C.NH} \\ \dot{\mathrm{C}}\mathrm{O.NH.}\dot{\mathrm{C}}\mathrm{ : N}\end{matrix} > \mathrm{CO}$ (E. FISCHER[3]), is found in the muscles, liver, spleen, pancreas, kidneys, testicles, carp-sperm, thymus, and brain. It occurs in small quantities as a physiological constituent of urine, and it has been found rarely as a urinary sediment or calculus. It was first observed in such a stone by MARCET. Xanthin is found in larger amounts in a few varieties of guano (Jarvis guano).

Xanthin is amorphous, or forms granular masses of crystals. It is very slightly soluble in water, in 14,151–14,600 parts at + 16° C., and in 1300–1500 parts at 100° C. (ALMÉN[4]). It is insoluble in alcohol or ether, but is dissolved by alkalies or acids. With hydrochloric acid it gives a crystalline, difficultly soluble combination. With very little caustic soda it gives a readily crystallizable combination, which is easily dissolved by an excess of alkali. Xanthin dissolved in ammonia gives with silver nitrate an insoluble, gelatinous precipitate of xanthin silver. This precipitate is dissolved by nitric acid, and by this means an easily soluble crystalline double combination is formed. A watery xanthin solution is precipitated on boiling with copper acetate. At ordinary temperatures xanthin is precipitated by mercuric chloride and by ammoniacal basic lead acetate. It is not precipitated with basic lead acetate alone.

When evaporated to dryness in a porcelain dish with nitric acid xanthin gives a yellow residue, which turns, on the addition of caustic soda, first red, and, after heating, purple-red. If we add some chloride of lime to some caustic soda in a porcelain dish and add the xanthin to this mixture, at first a dark green and then quickly a brownish halo forms around the xanthin grains and then disappears (Hoppe-Seyler). If xanthin be warmed in a small vessel on the water-bath with chlorine-water and a trace of nitric acid and evaporated to dryness, when the residue is exposed under a bell-jar to the vapors of ammonia a red or purple-violet color is produced (Weidel's reaction).

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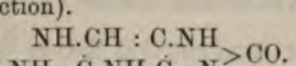
[1] Zur Kenntniss der Xanthinkörper. Inaug. Diss. Leipzig, 1893.

[2] Zeitschr. f. physiol. Chem., Bd. 18.

[3] Annal. d. Chem., Bd. 215.

[4] Journ. f. prakt. Chem., Bd. 96.



Guanin, $C_5H_5N_5O = \begin{array}{l} NH.CH : C.NH \\ NH : \dot{C}.NH.\dot{C} : N \end{array} > CO.$ Guanin is found in organs rich in cells, such as the liver, spleen, pancreas, testicles, and in salmon-sperm. It is further found in the muscles (in very small amounts), in the scales and in the air-bladder of certain fishes as iridescent crystals of guanin lime; in the *retina epithelium* of fishes, in guano, and in the excrement of spiders it is found as chief constituent. It also occurs in human and pig urine. Under pathological conditions it has been found in leucæmic blood, and in the muscles, ligaments, and articulations of pigs with guanin gout.

Guanin is a colorless, ordinarily amorphous powder which may be obtained as small crystals by allowing its solution in concentrated ammonia to spontaneously evaporate. It is nearly insoluble in water, alcohol, and ether. It is easily dissolved by mineral acids and alkalies, but it dissolves with great difficulty in ammonia. According to Wulff[1] 100 c.c. of cold ammonia solution containing 1, 3, and 5% NH_3 dissolve 9, 15, and 19 milligrammes guanin respectively. The solubility is relatively increased in hot ammonia solution. The hydrochloric-acid salt readily crystallizes, and this has been recommended by Kossel[2] in the microscopical detection of guanin on account of its behavior to polarized light. Very dilute guanin solutions are precipitated by both picric acid and metaphosphoric acid. These precipitates may be used in the quantitative estimation of guanin. The silver combination dissolves with difficulty in boiling nitric acid, and on cooling the double combination crystallizes out readily. Guanin acts like xanthin in the nitric-acid test, but gives with alkalies on heating a more bluish-violet color. A warm solution of guanin hydrochloride gives with a cold saturated solution of picric acid a yellow precipitate consisting of silky needles (CAPRANICA [1]). With a concentrated solution of potassium bichromate a guanin solution gives a crystalline, orange-red precipitate, and with a concentrated solution of potassium ferricyanide a yellowish-brown, crystalline precipitate (CAPRANICA).

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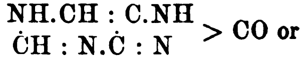
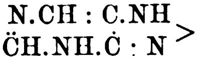
[1] Zeitschr. f. physiol. Chem., Bd. 17, S. 505.

[2] Ueber die chem. Zusammensetzung der Zelle. Verhandl. der physiol. Gesellsch. zu Berlin, 1890–1891, Nos. 5 and 6.

The composition of these and other guanin combinations have been studied by KOSSEL and WULFF.[2]

Hypoxanthin or SARKIN, $C_5H_4N_4O =$  $\begin{array}{l}\text{NH.CH : C.NH}\\ \dot{\text{C}}\text{H : N.}\dot{\text{C}}\text{ : N}\end{array} > \text{CO}$ or  $\begin{array}{l}\text{N.CH : C.NH}\\ \dot{\text{C}}\text{H.NH.}\dot{\text{C}}\text{ : N}\end{array} > \text{CO}$ (KRÜGER [3]). This body is found in the same tissues as xanthin. It is especially abundant in the sperm of the salmon and carp. Hypoxanthin occurs also in the marrow and in very small quantities in normal urine, and, as it seems, also in milk. It is found in rather considerable quantities in the blood and urine in leucæmia.

Hypoxanthin forms very small colorless crystalline needles. It dissolves in 300 parts cold and 78 parts boiling water. It is nearly insoluble in alcohol, but is dissolved by acids and alkalies. The combination with hydrochloric acid is crystalline, but is more soluble than the corresponding xanthin combination. This combination is easily soluble in dilute alkalies and ammonia. The silver combination dissolves with difficulty in boiling nitric acid. On cooling a mixture of two hypoxanthin silver, nitrate combinations not having a constant composition separates out. On treating this mixture with ammonia and excess of silver nitrate in the warmth, a hypoxanthin silver combination is formed, which when dried at 120° C. has a constant composition, $2(C_5H_2Ag_2N_4O)H_2O$, and which is used in the quantitative estimation of hypoxanthin. Hypoxanthin picrate is soluble with difficulty, but if a boiling-hot solution of the same is treated with a neutral or only faintly acid solution of silver nitrate the hypoxanthin is nearly quantitatively precipitated as the compound $C_5H_3AgN_4O.C_6H_2(NO_2)_3OH$. Hypoxanthin does not form any combination with metaphosphoric acid. When treated, like xanthin, with nitric acid, it yields a nearly colorless residue which on warming with alkali does not turn red. Hypoxanthin does not give WEIDEL's reaction. After the action of hydrochloric acid and zinc a hypoxanthin solution becomes first ruby-red and then brownish red in color on the addition of an excess of alkali (KOSSEL[1]).

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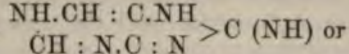
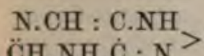
[1] Zeitschr. f. physiol. Chem., Bd. 4, S. 233.

[2] *Ibid.*, Bd. 17, S. 468.

[3] *Ibid.*, Bd. 18, S. 459.

Adenin,[2] $C_5H_5N_5$ =  $\begin{matrix}\mathrm{NH.CH : C.NH} \\ \dot{\mathrm{C}}\mathrm{H : N.C : N}\end{matrix}>\mathrm{C\ (NH)}$ or  $\begin{matrix}\mathrm{N.CH : C.NH} \\ \ddot{\mathrm{C}}\mathrm{H.NH.}\dot{\mathrm{C}}\mathrm{ : N}\end{matrix}>\mathrm{C\ (NH)}$, KRÜGER,[3] was first found by KOSSEL in the pancreas. It occurs in all nucleated cells, but in greatest quantities in the sperm of the carp and in the thymus. Adenin has also been found in leucæmic urine (STADTHAGEN[4]). It may be obtained in large quantities from tea-leaves. Adenin crystallizes with 3 mol. water of crystallization in long needles which become opaque gradually in the air, but much more rapidly when warmed. If the crystals are warmed slowly with a quantity of water insufficient for solution, they become suddenly cloudy at 53° C., a characteristic reaction for adenin. It dissolves in 1086 parts cold water, but is easily soluble in warm. It is insoluble in ether, but somewhat soluble in hot alcohol. Adenin is easily soluble in acids and alkalies. It is more easily soluble in ammonia solution than guanin, but less soluble than hypoxanthin. The silver combination of adenin is difficultly soluble in warm nitric acid, and deposits on cooling as a crystalline mixture of adenin silver nitrates. With picric acid adenin forms a compound, $C_5H_5N_5.C_6H_2(NO_2)_3OH$, which is very insoluble and which separates more readily than the hypoxanthin picrate and which can be used in the quantitative estimation of adenin. We also have an adenin mercury picrate. Adenin gives a precipitate with metaphosphoric acid, if the solution is not too dilute, which dissolves in an excess of the acid. Adenin hydrochloride gives with gold chloride a double combination which consists in part of leaf-shaped aggregations and in part of cubical or prismatic crystals, often with rounded corners. This compound is used in the microscopic detection of adenin. With the nitric-acid test and with WEIDEL's reaction adenin acts in the same way as hypoxanthin. The same is true for its behavior to hydrochloric acid and zinc and subsequent addition of alkali.

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[1] Zeitschr. f. physiol. Chem., Bd. 12.

[2] See Kossel, *ibid.*, Bdd. 10 and 12.

[3] *Ibid.*, Bd. 18, S. 459.

[4] Virchow's Arch., Bd. 109.

The principle for the preparation, detection, and the quantitative estimation of the four above-described xanthin bodies in organs and tissues is, according to KOSSEL and his pupils, as follows: The finely divided organ or tissue is boiled for three or four hours with sulphuric acid of about 5 p. m. The filtered liquid is freed from proteid by basic lead acetate, and the new filtrate is treated with sulphuretted hydrogen to remove the lead, again filtered, concentrated, and, after adding an excess of ammonia, precipitated with ammoniacal silver nitrate. The silver combination (with the addition of some urea to prevent nitrification) is dissolved in not too large a quantity of boiling nitric acid of sp. gr. 1.1, and this solution filtered boiling hot. On cooling the xanthin silver remains in the solution, while the double combination of guanin, hypoxanthin, and adenin crystallizes out. The xanthin silver may be precipitated from the filtrate by the addition of ammonia, and the xanthin set free by means of sulphuretted hydrogen. The three above-mentioned silver nitrate combinations are decomposed in water with ammonium sulphide and heat; the silver sulphide is filtered, the filtrate concentrated, saturated with ammonia, and digested on the water-bath. The guanin remains undissolved, while the other two bases pass into solution. A part of the guanin is still retained by the silver sulphide, and may be liberated by boiling it with dilute hydrochloric acid and then saturating the filtrate with ammonia. When the above filtrate, containing the adenin and hypoxanthin, which has been, if necessary, freed from ammonia by evaporation, is allowed to cool, the adenin separates, while the hypoxanthin remains in solution. According to BALKE[1] we can to advantage precipitate the xanthin bases with copper sulphate and hydroxylamin as above mentioned and then further separate the bodies.

The prominent points in the above method are made use of in the quantitative estimation of xanthin bases. The xanthin is weighed as xanthin silver. The three silver nitrate combinations are transformed into the corresponding silver combination by the addition of ammonia with silver nitrate and then this acted on, after thorough washing, by ammonium sulphide. Guanin is weighed as such. The ammoniacal filtrate containing the adenin and hypoxanthin, and which must not be mixed with the hydrochloric-acid extract of the silver sulphide, is neutralized and treated with a cold concentrated solution of sodium picrate until the solution is pronouncedly yellow. The adenin picrate is filtered off immediately, washed on the filter with water, dried at above 100° C., and weighed. The filtrate containing the hypoxanthin is gradually treated, while boiling hot, with silver nitrate, and when cold treated with silver nitrate to see whether precipitation has been complete.

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[1] Zur Kenntnisse der Xanthinkörper. Inaug. Diss. Leipzig, 1893.

The hypoxanthin picrate is washed, dried at 100° C., and weighed. In regard to the composition of these compounds see pages 106 and 107. This method of separating adenin and hypoxanthin presupposes that the liquid does not contain any hydrochloric acid.

The above method of separation with ammonia does not give exact results on account of the not inconsiderable solubility of guanin in warm ammonia. According to KOSSEL and WULFF[1] the guanin may therefore be precipitated from sufficiently dilute solutions by an excess of metaphosphoric acid and the nitrogen determined in the washed precipitate by KJELDAHL'S method. The adenin and hypoxanthin may be precipitated from the filtrate by ammoniacal silver nitrate. The silver compound is decomposed with very dilute hydrochloric acid and the adenin separated from the hypoxanthin according to the suggestion of BRUHNS.[2]

Mineral bodies are never-failing constituents of the cell. These mineral bodies are potassium, sodium, calcium, magnesium, iron, phosphoric acid, and chlorine. In regard to the alkalies we find in general in the animal organism that the sodium combinations are more abundant in the fluids, and the potassium combinations in the form-constituents and in the protoplasm. Corresponding to this the cell contains potassium, chiefly as phosphate, while the sodium and chlorine combinations occur less abundantly. According to the ordinary views the potassium combinations, especially the potassium phosphate, are of the greatest importance for the life and development of the cell, even though we do not know the nature of the importance.

In regard to the phosphoric acid there seems to be no doubt that its importance lies chiefly in that it takes part in the formation of nucleins and thereby indirectly makes possible the processes of growth and division, which are dependent upon the cell nucleus. LOEW[3] has shown, by means of cultivation experiments on algae Spirogyra, that only on the supplying of phosphates (in his experiments potassium phosphate) was the nutrition of the cell nucleus made possible, and thereby the growth and division of the cells. The cells of the Spirogyra can be kept alive and indeed produce starch and proteids for some time without a supply of phosphates, but its growth and propagation suffers. Phosphoric acid is also without doubt of importance in the formation of the lecithins.

Iron seems to occur especially in the nucleus, because the nucleins are very rich therein. The regular occurrence of earthy phosphates in all cells and tissues, as also the difficulty or rather the impossibility of separating these bodies from the protein bodies without modifying them, leads to the supposition that these mineral bodies are of unknown but nevertheless great importance for the life of the cell, as well as the chemical processes going on within them.

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[1] Zeitschr. f. physiol. Chem., Bd. 17.

[2] *Ibid.*, Bd. 14, S. 559.

[3] Biologisches Centralblatt, Bd. 11, 1891, S. 269.

CHAPTER VI.

THE BLOOD.

THE blood is to be considered from a certain standpoint as a fluid tissue, and it consists of a transparent liquid, the *blood-plasma*, in which a vast number of solid particles, the *red* and *white blood-corpuscles* (and the *blood-plates*) are suspended.

Outside of the organism the blood, as is well known, coagulates more or less quickly; but this coagulation is accomplished generally in a few minutes after leaving the body. All varieties of blood do not coagulate with the same degree of rapidity. Some coagulate more quickly, others more slowly. Among the varieties of blood thus far investigated the blood of the horse coagulates most slowly. The coagulation may be more or less retarded by quickly cooling; and if we allow equine blood to flow directly from the vein into a glass cylinder which is not too wide and which has been cooled, and let it stand at 0° C., the blood may be kept fluid for several days. An upper, amber-yellow layer of plasma gradually separates from a lower, red layer composed of blood-corpuscles with only a little plasma. Between these we observe a whitish-gray layer, which consists of white blood-corpuscles.

The plasma thus obtained and filtered is a clear amber-yellow alkaline liquid which remains fluid for some time when kept at 0° C., but soon coagulates at the ordinary temperature.

The coagulation of the blood may be prevented in other ways. After the injection of peptone or, more correctly, albumose solutions into the blood (in the living dog), the blood does not coagulate on leaving the veins (FANO,[1] SCHMIDT-MÜLHEIM[2]). The plasma obtained from such blood by means of centrifugal force is called

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[1] Du Bois-Reymond's Archiv, 1881, S. 277.

[2] *Ibid.*, 1880.

"*peptone-plasma.*" The coagulation of the blood of warm-blooded animals is prevented by the injection of an effusion of the mouth of the officinal leech into the blood-current (HAYCRAFT[1]). If the blood-circulation of a dog is cut off from the liver and intestine and the blood allowed to flow only through the head and the viscera of the thoracic cavity, the coagulation property of the blood is destroyed (PAWLOW, BOHR[2]). The statement as to the non-coagulability of the blood after the excision of the liver and abnominal cavity could not be confirmed by CONTEJEAN.[3] If we allow the blood to flow directly, while we stir it, into a neutral salt solution—best a saturated magnesium-sulphate solution (1 vol. salt solution and 3 vols. blood)—we obtain a mixture of blood and salt which remains uncoagulated for several days. The blood-corpuscles which, because of their adhesiveness and elasticity, would otherwise pass easily through the pores of the filter-paper are made solid and stiff by the salt, so that they may be easily filtered. The plasma thus obtained, which does not coagulate spontaneously, is called "*salt-plasma.*"

An especially good method of preventing coagulation of blood consists in drawing the blood into a dilute solution of potassium oxalate, so that the mixture contains 0.1% oxalate (ARTHUS and PAGÈS[4]). The soluble calcium salts of the blood are precipitated by the oxalate, and hence the blood loses its coagulability.

On coagulation there separates in the previously fluid blood an insoluble or a very difficultly soluble albuminous substance, *fibrin.* When this separation takes place without stirring, the blood coagulates to a solid mass which, when carefully severed from the sides of the vessel, contracts, and a clear, generally yellow-colored liquid, the *blood-serum*, exudes. The solid coagulum which encloses the blood-corpuscles is called the *blood-clot* (placenta sanguinis). If the blood is beaten during coagulation, the fibrin separates in elastic threads or fibrous masses, and the *defibrinated blood* which separates is sometimes called *cruor*,[5] and consists of blood-corpuscles and blood-serum. Defibrinated blood consists of blood-corpuscles and serum, while uncoagulated blood consists of blood-corpuscles and blood-plasma. The essential chemical difference between blood-serum, and blood-plasma is that the blood-serum does not contain the mother-substance of fibrin, the fibrinogen, which exists in the blood-plasma, and the serum is proportionally richer in another body, the fibrin ferment (see page 116).

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[1] Proc. physiol. Soc., 1884, p. 13, and Arch. f. exp. Pathol. und Pharm., 1884, Bd. 18.

[2] Centralbl. f. Physiol., 1888, No. 11.

[3] Arch. de Physiol., Sér. 5, Tome 7.

[4] *Ibid.*, Tome 2, 1890, and Compt. rend., 1891, Tome 112, No. 4.

[5] The name *cruor* is used in different senses. We sometimes understand thereby only the blood when coagulated to a red solid mass, in other cases the blood-clot after the separation of the serum, and lastly the sediment consisting

I. Blood-plasma and Blood-serum.

The Blood-plasma.

In the coagulation of the blood a chemical transformation takes place in the plasma. A part of the proteids separates as insoluble fibrin. The albuminous bodies of the plasma must therefore be first described. They are, as far as we know at present, *fibrinogen*, *serglobulin*, and *seralbumin*.

Fibrinogen occurs in blood-plasma, chyle, lymph, and in certain transudations and exudations.[1] It has the general properties of the globulins, but differs from other globulins as follows: In a moist condition it forms white flakes which are soluble in dilute common-salt solutions, and which easily conglomerate into tough, elastic masses or lumps. The solution in NaCl of 5-10% coagulates on heating to + 52° to 55° C., and the faintly alkaline or nearly neutral weak salt solution coagulates at + 56° C., or at exactly the same temperature at which the blood-plasma coagulates. Fibrinogen solutions are precipitated by an equal volume of a saturated common-salt soluion, and are completely precipitated by adding an excess of NaCl in substance (thus differing from serglobulin). It differs from myosin of the muscles, which coagulates at about the same temperature, and from other albuminous bodies, in the property of being converted into fibrin under certain conditions. Fibrinogen has a strong decomposing action on hydrogen peroxide.[2] It is quickly made insoluble by precipitation with water or with

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of red blood-corpuscles which is obtained from defibrinated blood by means of centrifugal force or by letting it stand.

[1] The question as to the occurrence of other fibrinogens (WOOLDRIDGE) will be spoken of in connection with the complete discussion of the coagulation of the blood. (See further on.)

[2] In regard to fibrinogen the reader is referred to the author's investigations. Pflüger's Archiv, Bdd. 19 and 22.

dilute acids. Its specific rotation is $\alpha(D) = -52.5°$ according to MITTELBACH.[1]

Fibrinogen may be easily separated from the salt-plasma by precipitation with an equal volume of a saturated NaCl solution. For further purification the precipitate is pressed, redissolved in an 8% salt solution, the filtrate precipitated by a saturated-salt solution as above, and after precipitating in this way three times the precipitate at last obtained is pressed between filter-paper and finely divided in water. The fibrinogen dissolves with the aid of the small amount of NaCl contained in itself, and the solution may be made salt-free by dialysis with very faintly alkaline water. From transudations we ordinarily obtain a fibrinogen which is strongly contaminated with lecithin and which can hardly be purified without decomposing. The method for the detection and quantitative estimation of fibrinogen in a liquid is based on its property of yielding fibrin on the addition of a little blood, of serum, or of fibrin ferment.

The fibrinogen stands in close relation to its transformation-product, the fibrin.

Fibrin is the name of that proteid body which separates on the so-called spontaneous coagulation of blood, lymph, and transudations, as also in the coagulation of a fibrinogen solution after the addition of serum or fibrin ferment (see below).

If the blood is beaten during coagulation, the fibrin separates in elastic, fibrous masses. The fibrin of the blood-clot may be beaten to small, less elastic, and not particularly fibrous lumps. The typical, fibrous, and elastic white fibrin, after washing, stands in regard to its solubility close to the coagulated proteids. It is insoluble in water, alcohol, or ether. It expands in hydrochloric acid of 1 p. m., as also in caustic potash or soda of 1 p. m., to a gelatinous mass, which dissolves at the ordinary temperature only after several days, but at the temperature of the body it dissolves more readily but still slowly. Fibrin expands in a 5–10% solution of common salt or saltpetre, but only dissolves very slowly at ordinary temperature, while at 40° C. it dissolves more readily. At present we cannot positively state what action the presence of micro-organisms or contaminating enzymes have on this solution. According to ARTHUS and HUBER,[2] and also lately to DARESTE,[3] there is no doubt of the solubility of fibrin in neutral salt solutions without the action of micro-organisms. According to GREEN[1] two globulins are formed in this solution of fibrin. Fibrin decomposes hydrogen peroxide, but this property is destroyed by heating or by the action of alcohol.

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[1] Zeitschr. f. physiol. Chem., Bd. 19.

[2] Arch. de Physiol., Sér. 5, Tome 5.

[3] *Ibid.*, Tome 7.

What has been said of the solubility of fibrin relates only to the typical fibrin obtained from the arterial blood of mammals or man by whipping and washing first with water and with common-salt solution, and then with water again. The blood of various kinds of animals yields fibrin with somewhat different properties, and according to FERMI[2] pig-fibrin dissolves much more readily in hydrochloric acid of 5 p. m. than ox-fibrin. Fibrins of varying purity or originating from blood from different parts of the body have unlike solubilities.

The fibrin obtained by beating the blood and purified as above described is always contaminated by enclosed blood-corpuscles or remains thereof, and also by lymphoid cells. It can only be obtained pure from filtered plasma or filtered transudations. For the pure preparation, as well as for the quantitative estimation of fibrin, the spontaneously coagulating liquid is at once, or the non-spontaneously coagulating liquid only after the addition of blood-serum or fibrin ferment, thoroughly beaten with a whale-bone, and the separated coagulum is washed first in water, and then with a 5% common-salt solution, and again with water, and lastly extracted with alcohol and ether. If the fibrin is allowed to stand in contact with the blood from which it was formed for some time, it partly dissolves (fibrinolysis—DASTRE[3]). This fibrinolysis must be prevented in the quantitative estimation of fibrin (DASTRE).

A pure fibrinogen solution may be kept at the ordinary temperature until putrefaction begins without showing a trace of fibrin coagulation. But if to this solution we add a water-washed fibrin-clot or a little blood-serum, it immediately coagulates and may yield perfectly typical fibrin. The transformation of the fibrinogen into fibrin requires the presence of another body contained in the blood-clot and in the serum. This body, whose importance in the coagulation of fibrin was first observed by BUCHANAN,[4] was later rediscovered by ALEXANDER SCHMIDT[5] and designated "*fibrin-*

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[1] Journal of Physiol., Vol. 8, p. 512.

[2] Zeitschr. f. Biologie, Bd. 28, S. 229.

[3] Archives de Physiol. (5), Tome 5, No. 3, and Tome 6, No. 4, p. 670.

[4] London Med. Gazette, 1845, p. 617. Cit. by Gamgee, Journal of Physiol., 1879.

[5] Pflüger's Archiv, Bd. 6, S. 413.

ferment." The nature of this enzymotic body has not been ascertained. Although many investigators, especially English, consider fibrin ferment as a globulin, still more recent experiments of PEKELHARING,[1] WRIGHT,[2] and LILIENFELD[3] show that it is a nucleoalbumin or perhaps a nucleoproteid. Fibrin ferment, which is now called *thrombin* by ALEX. SCHMIDT,[4] is produced, according to PEKELHARING, by the action of soluble calcium salts on a preformed zymogen existing in the non-coagulated plasma. SCHMIDT admits of the presence of such a mother-substance of the fibrin ferment in the blood and calls it prothrombin. The zymogen as well as the fibrin ferment is less soluble in an excess of acetic acid than the globulins, and yields a nuclein or a pseudonuclein on peptic digestion. Thrombin corresponds to other enzymes in that the very smallest amount of it produces an action and its solution becomes inactive on heating. It is most active at about 40° C. The zymogen, according to PEKELHARING, is destroyed at about 65° C., while the ferment is destroyed at about the same or a little higher temperature, 70–75° C.

The isolation of the fibrin-ferment has been tried in several ways. Ordinarily it may be prepared by the following method proposed by ALEX. SCHMIDT: Precipitate the serum or defibrinated blood with 15–20 vols. of alcohol and allow it to stand a few months. The precipitate is then filtered and dried over sulphuric acid. The ferment may be extracted from the dried powder by means of water.

A globulin-free thrombin solution may be prepared as follows, according to the author. The globulins are separated from ox serum by complete saturation with magnesium sulphate, [illegible]

[illegible]

Thrombin can be precipitated from this solution, according to PEKELHARING,[1] by the proper addition of acetic acid. According to this investigator, it is best to dialyze the above filtrate saturated with $MgSO_4$ and then precipitate with acetic acid. He has been able to obtain thrombin directly from the blood-serum by diluting with water and adding acetic acid until the serglobulin, which first precipitates, is at least in great part redissolved. The thrombin is purified by repeated solution in alkaline water and reprecipitating with acetic acid.

If a fibrinogen solution containing salt, as above prepared, is treated with a solution of "fibrin-ferment," it coagulates at the ordinary temperature more or less quickly and yields a typical fibrin. Besides the fibrin ferment the presence of neutral salts is necessary, for without them ALEX. SCHMIDT[2] has shown the coagulation of fibrin does not take place. The presence of soluble calcium salts is likewise an essential condition for the formation of fibrin (ARTHUS and PAGÈS, PEKELHARING), and the fibrin separated always contains calcium. The quantity of fibrin obtained on coagulation is always smaller than the amount of fibrinogen from which the fibrin is derived, and we always find a small amount of protein substance in the solution. It is therefore not improbable that the coagulation of fibrin, in accordance with the views of DENIS, is a splitting process in which the soluble fibrinogen is split into an insoluble albuminous body, the fibrin, which forms the chief mass, and a soluble protein substance which is only formed in small amounts. We find a globulin-like substance which coagulates at about + 64° C. in blood-serum as well as in the serum from coagulated fibrinogen solutions. This substance is called *fibrin-globulin* by the AUTHOR.[3] The question whether this substance exists in the fibrinogen solution as contamination or is formed as a splitting product has not been positively decided.

The lime-salts, as above stated, are a necessary factor in the coagulation. According to PEKELHARING,[4] they act as follows: The fibrin-ferment or thrombin is a calcium combination of the zymogen, the prothrombin. In coagulation the calcium is transferred to the fibrinogen by the thrombin, forming insoluble fibrin containing calcium. The thrombin is hereby reconverted into prothrombin, which takes up more calcium, being converted into thrombin again, which then gives up its calcium to a new portion of fibrinogen, and so on. The process has great similarity to the formation of ether from alcohol by sulphuric acid.

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[1] L. c.

[2] Pflüger's Archiv, Bd. 11, S. 291–304; also Bd. 13, S. 103.

[3] *Ibid.*, Bd. 22.

[4] Verhandel. d. kon. Akad. d. Wettensch. te Amsterdam, Deel 1, No. 3, 1892.

LILIENFELD[1] has described his experiments and views in an extensive memoir. According to him the fibrinogen may be split by acetic acid, and also by the nuclein substances of the leucocytes (these also act in alkaline solution), into a proteid body, which is precipitated readily, *thrombosin*, and an albumose-like substance, which gives the biuret reaction and which retards coagulation. Thrombosin passes into fibrin in the presence of soluble calcium salts, without further addition inasmuch as fibrin is nothing but the calcium combination of thrombosin. The above cleavage of fibrinogen into thrombosin and a soluble proteid substance may also take place in the absence of calcium salts, and these are only necessary for the separation of the calcium combination of thrombosin, *i.e.*, fibrin. Fibrin-ferment, which is a globulin according to LILIENFELD, is not a precursor but a product of the coagulation. The coagulation process is considered by LILIENFELD and most investigators as a cleavage of the fibrinogen, and the essential difference between his theory and the others consists in that the coagulation exciter is not the fibrin-ferment but a nucleoproteid which is the leuconuclein derived from the nucleohiston by cleavage.

HALLIBURTON and BRODIE[2] have raised an objection to the statement of PEKELHARING as to the identity of fibrin-ferment with a nucleoproteid or its calcium combination occurring in the blood-plasma. PEKELHARING[3] has repudiated this in a recent article. He has shown, in opposition to the views of HALLIBURTON and LILIENFELD, that the fibrin-ferment yields nuclein in careful pepsin digestion, hence it must be a nucleoproteid. In a work which appeared after the death of ALEX. SCHMIDT[4] he has given his position on the work of other investigators in this field, but as this extensive work is chiefly of a critical nature we cannot discuss it.

According to DOGIEL and HOLZMANN[5] the fibrin coagulation consists in an oxidation of fibrinogen. The relationship of oxygen to the coagulation is indeed not clear, and that it has a certain influence on the coagulation cannot be denied; still, as coagulation may take place in the absence of free oxygen, the above view does not seem to be based on sufficient fact.

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[1] Zeitschr. f. physiol. Chem., Bd. 20.

[2] Journal of Physiol , Vol. 17.

[3] Centralbl. f. Physiol., 1895, Heft 3.

[4] Weitere Beiträge zur Blutlehre. Wiesbaden, 1895.

[5] Compt. rend. d. congrès internat. des sciences médicales à Copenhague, 1884, Tome 1, p. 135

Although the processes of coagulation are still not clear, nevertheless they consist essentially in the conversion of the fibrinogen of the plasma into fibrin. The coagulation of the blood is a much more complicated process than the coagulation of a fibrinogen solution, inasmuch as the first involves other important questions, as, for instance, the reason for the blood remaining fluid in the body, the origin of the fibrin-ferment, and the importance of the form-elements in the coagulation. A fuller discussion of the various hypotheses and theories concerning the coagulation of the blood must therefore be given later.

Serglobulin, also called *paraglobulin* (KÜHNE[1]), *fibrinoplastic substance* (ALEX. SCHMIDT[2]), *serum-casein* (PANUM[3]), occurs in the plasma, serum, lymph, transudations and exudations, in the white and red corpuscles, and probably in many animal tissues and form-elements, though in small quantities. It is also found in the urine in many diseases.

Serglobulin is without doubt not an individual substance, but consists of a mixture of two or more protein bodies which cannot be completely and positively separated from each other. Under these circumstances the statements in regard to the properties of the serglobulins is naturally somewhat uncertain. According to our present knowledge it has the following properties:[4]

Serglobulin has the general properties of the globulins. In a moist condition it forms a snow-white flaky mass neither tough nor elastic. The essential differences between serglobulin and fibrinogen are the following: Serglobulin solutions are only incompletely precipitated by adding NaCl to saturation, and not precipitated at all by an equal volume of a saturated common-salt solution. The coagulation temperature is, with 5–10% NaCl in solution, + 75° C. It is completely precipitated by $MgSO_4$ in substance added to saturation, as also by an equal volume of a saturated solution of ammonium sulphate. The specific rotatory power, according to FRÉDERICQ,[1] for serglobulin (from ox-blood) solutions containing salt is $\alpha(D) = -47.8°$.

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[1] Lehrbuch d. physiol. Chem. Leipzig, 1866–68.

[2] Arch. f. Anat. u. Physiol., 1861, S. 545, and 1862, S. 428.

[3] Virchow's Archiv, Bd. 4.

[4] See Hammarsten, Ueber Paraglobulin, Pflüger's Archiv, Bdd. 17 and 18.

According to K. MÖRNER[2] serglobulin yields a reducing substance on boiling with a dilute acid. The question whether the substance we have heretofore called serglobulin is a glycoproteid or whether it is a mixture of globulin with a glycoproteid has not been positively decided up to the present time.

Serglobulin may be easily separated as a fine flocculent precipitate from blood-serum by neutralizing or making faintly acid with acetic acid and then diluting with 10–20 vols. of water. For further purification this precipitate is dissolved in dilute common-salt solution, or in water by the aid of the smallest possible amount of alkali, and then reprecipitated by diluting with water or by the addition of a little acetic acid. The serglobulin may also be separated from the serum by means of magnesium or ammonium sulphate; in these cases it is difficult to completely remove the salt by dialysis. The serglobulin from blood-serum is always contaminated by lecithin and so-called fibrin-ferment. A serglobulin free from fibrin-ferment may be prepared from ferment-free transudations, as sometimes from hydrocele fluids, and this shows that serglobulin and fibrin-ferment are different bodies. For the detection and the quantitative estimation of serglobulin we may use the precipitation by magnesium sulphate added to saturation (AUTHOR[3]), or by an equal volume of a saturated *neutral* ammonium sulphate solution (HOFMEISTER and KAUDER and POHL[4]). In the quantitative estimation the precipitate is collected on a weighed filter, washed with the salt solution employed, dried with the filter at about 115° C., then washed with boiling-hot water, so as to completely remove the salt, extracted with alcohol and ether, dried, weighed and burnt to determine the ash.

Seralbumin is found in large quantities in blood-serum, blood-plasma, lymph, transudations, and exudations. Probably it also occurs in other animal liquids and tissues. The proteids which pass into the urine under pathological conditions consists largely of seralbumin.

In the dry state seralbumin forms a transparent, gummy, brittle, hygroscopic mass, or a white powder which may be heated to 100° C. without decomposing. Its solution in water gives the ordinary reactions for albumins; the specific rotatory power for seralbumin free from paraglobulin, obtained from human transudations, is, according to STARK,[1] $\alpha(D) = -62.6°$ to $-64.6°$. The coagulation temperature of a seralbumin solution is + 70° to + 75° C., according to most authorities, but this varies to a great extent with a varying concentration and amount of salt (STARK). A 1-2% seralbumin solution may, in the presence of very little NaCl, coagulate at + 50° C. or below; in the presence of 5% NaCl it coagulates at + 75° to + 90° C. By the careful addition of acid the coagulation temperature may be lowered; by the addition of alkali it may be raised. In blood-serum from certain animals and in human transudations HALLIBURTON[2] found the coagulation to take place on heating to the following temperatures: + 70° to 73° C.; 77° to 78° C.; and 82° to 85° C. He therefore considers the seralbumin as a mixture of three albumins, α, β, and γ, which coagulate at the three points mentioned. In cold-blooded animals he found only the α-albumin. GURBER[3] has prepared crystallized proteid from blood-serum of the horse, which seems to correspond to three different seralbumins.

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[1] Bull. Acad. Roy. de Belg. (2), Tome 50.

[2] Centralbl. f. Physiol., 1893, No. 20.

[3] Pflüger's Archiv, Bd. 17, S. 447.

[4] Arch. f. exp. Path. u. Pharm., Bd. 20, S. 411 and 426.

Seralbumin differs from the albumin of the white of the hen's egg in the following particulars: it is more lævogyrate; the precipitate formed by hydrochloric acid easily dissolves in an excess of the acid; is rendered less insoluble by alcohol; and lastly it acts differently inside of the organism. If egg-albumin is introduced into the blood system it passes into the urine, while seralbumin does not. A solution of seralbumin positively free from mineral bodies has never yet been prepared. A solution as poor as possible in salts does not coagulate either on boiling or on the addition of alcohol. After the addition of a little common salt it coagulates in both cases.

In preparing seralbumin, first remove the globulins according to JOHANSSON,[4] by saturating with magnesium sulphate at about + 30° C., and filtering at the same temperature. The cooled filtrate is separated from the crystallized salt and is treated with acetic acid so that it contains about 1%. The precipitate formed is filtered, pressed, dissolved in water with the addition of alkali to neutral reaction, and the solution freed from salt by dialysis. The seralbumin may also be separated from the filtrate saturated with magnesium sulphate by adding sodium sulphate to saturation at about + 40° C. (STARK[1]). The pressed precipitate is also in this case dissolved in water and the solution freed from salt by dialysis. The albumin may be obtained in a solid form from the dialyzed solution either by evaporating the solution to dryness at gentle heat or by precipitating with alcohol, which must be removed quickly. In the detection and quantitative estimation of seralbumin, the filtrate from the globulins which have been removed by magnesium sulphate is heated to boiling, after the addition of a little acetic acid if necessary. The simplest way is to consider the difference between the total proteids and the globulins as seralbumin.

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[1] Maly's Jahresbericht, Bd. 11.

[2] Journal of Physiol., Vols. 5 and 7.

[3] Sitzungsber. d. Würzb. phys. med. Gesellsch., 1894.

[4] Zeitschr. f. physiol. Chem., Bd. 9, S. 317.

Summary of the elementary composition of the above mentioned and described albuminous bodies:

	C	H	N	S	O	
Fibrinogen..........	52.93	6.90	16.66	1.25	22.26	(HAMMARSTEN)
Fibrin..............	52.68	6.83	16.91	1.10	22.48	"
Fibrin-globulin.....	52.70	6.98	16.06	...	....	"
Serglobulin.........	52.71	7.01	15.85	1.11	23.32	"
Seralbumin (1)......	53.06	6.85	16.04	1.80	22.26	"
Seralbumin (2)......	52.25	6.65	15.88	2.25	22.97	"

The seralbumin (2) came from a human exudation, and the other bodies from horse s blood. The fibrin was prepared from a filtered common-salt plasma.

The Blood-serum.

As above stated, the blood-serum is the clear liquid which is pressed out by the contraction of the blood-clot. It differs chiefly from the plasma in the absence of fibrinogen and an abundance of fibrin-ferment. Considered qualitatively the blood-serum contains the same chief constituents as the blood-plasma.

Blood-serum is a sticky liquid which is more alkaline than the plasma. The specific gravity in man is 1.027 to 1.032, average 1.028. The color is strongly or faintly yellow; in human blood-serum it is pale yellow with a shade towards green, and in horses it is often amber-yellow. The serum is ordinarily clear; after a meal it may be opalescent, cloudy, or milky white, according to the amount of fat contained in the food.

Besides the above-mentioned bodies, the following constituents are found in the blood-plasma or blood-serum:

Fat occurs from 1–7 p. m. in fasting animals. After partaking of food the amount is increased to a great extent. We also find *soaps* (HOPPE-SEYLER[1]), *cholesterin*, and *lecithin*.

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[1] Maly's Jahresber., Bd. 11.

Glucose seems to be a physiological constituent of the plasma. According to the investigations of ABELES, EWALD, KÜLZ, v. MERING,[2] and SEEGEN,[3] the sugar found in the plasma is glucose. OTTO[4] found in the plasma, besides glucose, another reducing, non-fermentable substance. The amount of glucose in the blood is about 1–1.5 p. m. OTTO found in human blood 1.18 p. m. glucose and 0.29 p. m. of the other reducing substance. According to JACOBSEN,[5] this is soluble in ether and is closely related to jecorin. The amount of glucose in the blood seems to be almost independent of the food; nevertheless after feeding with large quantities of glucose and dextrin BLEILE[6] observed a significant increase of glucose. If the amount is more than 3 p. m., according to CL. BERNARD,[7] the glucose passes into the urine, producing glycosuria. The different amounts of glucose in the blood from different vessels and under various conditions will be fully discussed later. The glycogen found in the blood does not seem to come from the plasma, but from the leucocytes.

BERNARD[8] has shown that the quantity of sugar in the blood diminishes more or less rapidly on leaving the veins. LÉPINE,[9] associated with BARRAL, has specially studied this decrease in the quantity of sugar and calls it *glycolysis*. LÉPINE and BARRAL as well as ARTHUS[10] have shown that this glycolysis takes place in the complete absence of micro-organisms.[11] It seems to be due to a soluble enzyme whose activity is destroyed by heating to + 54° C. This enzyme is derived, according to the above investigators, from the leucocytes and is, according to Lépine, delivered from the pancreas to the blood. According to Lépine[1] the pancreas contains a zymogen of the glycolytic enzyme occurring in the blood. This zymogen, which is converted into the enzyme by the action of sulphuric acid of 2 p. m. at 38° C., is nothing but the diastatic enzyme. Röhmann and Spitzer[2] and Spitzer,[3] who have shown the occurrence of a glycolysis under the influence of not only the blood but also various tissues, consider this, as first shown by Kraus,[4] a process of oxidation. This oxidation is brought about by the oxygen of the oxidation ferment occurring in the form-elements. According to Arthus and Colenbrander[5] this glycolysis is only a post-mortem process and not a vital one.

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[1] Zeitschr. f. physiol. Chem., Bd. 8.

[2] Du Bois-Reymond's Archiv, 1877, S. 379. This article contains numerous references.

[3] Pflüger's Archiv, Bd. 40.

[4] *Ibid.*, Bd. 35. Contains a good review of the literature of sugar in the blood.

[5] Centralbl. f. Physiol., 1892, Part 13.

[6] Du Bois-Reymond's Archiv, 1879, S. 67–69.

[7] Leçons sur le diabète. Paris, 1877.

[8] *Ibid.*

[9] Lyon médical, Tome 62 and 63, Compt. rendus, Tome 110, 112, and 113; Lépine, Le ferment glycolytique et la pathogénie du diabète (Paris, 1891), and Revue analytique et critique des travaux, etc., in Arch. de méd. exper. (Paris, 1892).

[10] Arch. de Physiol., July 1891 and April 1892.

[11] A critical review of the various methods of removing proteids from the blood in sugar estimations is given by Seegen, Centralbl. f. Physiol., 1892, Heft 17.

Blood-plasma contains an enzyme which converts starch and glycogen into sugar (Röhmann[6] and Bial[7]). This enzyme also occurs in the lymph, but not in the form-elements of the blood.

The sugar found in the serum by enzyme action is partly maltose or isomaltose and partly dextrose. These various sugars are produced in differing quantities in the various phases of the enzymotic processes. This is accounted for, by recent researches of Röhmann and C. Hamburger,[8] by the presence of two differing enzymes in the blood. One of these enzymes is diastase, which converts starch and glycogen into maltose. The other differs from invertin and, according to Röhmann, is probably an enzyme, identical with glucase, occurring in the plant kingdom, and which has the property of splitting maltose into dextrose.

Among the bodies which are found in the blood, and without doubt met with in smaller or greater amounts in the plasma, are to be mentioned *urea*, *uric acid* (found in human blood by Abeles[9]),

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[1] Compt. rend., Tome 120.

[2] Ber. d. deutsch. chem. Gesellsch., Bd. 28.

[3] Pflüger's Archiv, Bd. 60.

[4] Zeitschr. f. klin. Med., Bd. 21.

[5] Maly's Jahresber., Bd. 22.

[6] Ber. d. deutsch. chem. Gesellsch., Bd. 25, and Pflüger's Arch., Bd. 52.

[7] Ueber das diastatische Ferment des Lymph- und Blutserums. Inaug. Diss. Breslau, 1892. Contains also the older literature. See also Pflüger's Arch., Bdd. 52, 54, and 55.

[8] Ber. d. deutsch. chem. Gesellsch., Bd. 27, and Pflüger's Arch., Bd. 60.

[9] Wien. med. Jahrbücher, 1887.

ceratin, *carbamic acid*, *paralactic acid*, and *hippuric acid.* The quantity of urea depends upon the nutritive condition of the animal. During starvation SCHÖNDORFF[1] found a minimum of 0.348 p. m., and in the highest stages of urea formation a maximum of 1.529 p. m. Under pathological conditions the following bodies have been found: *xanthin bases*, *leucin*, *tyrosin*, and *biliary constituents*.

The *coloring matters* of the blood-serum are very little known. In equine blood-serum biliary coloring matters, bilirubin, besides other coloring matters, often occur. The yellow coloring matter of the serum seems to belong to the group of *luteins*, which are often called *lipochromes* or fat-coloring matters. From ox-serum KRUKENBERG[2] was able to isolate with amyl alcohol a so-called lipochrome whose solution shows two absorption-bands, of which one encloses the line *F* and the other lies between *F* and *G*.

The *mineral bodies* in serum and plasma are qualitatively, but not quantitatively, the same. A part of the calcium, magnesium, and phosphoric acid is removed on the coagulation of the fibrin. By means of dialysis, the presence of sodium chloride, which forms the chief mass or 60–70% of the total mineral bodies, also lime-salts, sodium carbonate, besides traces of sulphuric and phosphoric acids and potassium, may be directly shown in the serum. Traces of silicic acid, fluorine, copper, iron, manganese, and ammonia are claimed to have been found in the serum. As in most animal fluids, the chlorine and sodium are in the blood-serum in excess of the phosphoric acid and potassium (the occurrence of which in the serum is even doubted). The acids found in the ash are not sufficient to saturate the bases found, a condition which shows that a part of the bases is combined with organic substances, perhaps proteids.

The *gases* of the blood-serum, which consist chiefly of carbon dioxide with only a little nitrogen and oxygen, will be described when treating of the gases of the blood.

Because of the difficulty of obtaining plasma only a few analyses have been made. As an example the results of the analyses of the blood-plasma of the horse will be given below. The analysis No. 1 was made by HOPPE-SEYLER.[3] No. 2 is the average of the results of three analyses made by the AUTHOR.[1] The figures are given in 1000 parts of the plasma.

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[1] Pflüger's Archiv, Bd. 54.

[2] Sitzber. d. Jen. Gesellsch. f. Med., 1885.

[3] Cit. from v. Gorup-Besanez's Lehrbuch der physiol. Chem., 4. Aufl., p. 346.

	No. 1.	No. 2.
Water	908.4	917.6
Solids	91.6	82.4
Total proteids	77.6	69.5
Fibrin	10.1	6.5
Globulin	....	38.4
Seralbumin	....	24.6
Fat	1.2	12.9
Extractive substances	4.0	
Soluble salts	6.4	
Insoluble salts	1.7	

As an example of the composition of blood-serum with special regard to the relationship of the different proteids to each other, the following analyses are given. The results are in 1000 parts.

Serum from	Solids.	Total Albuminous Bodies.	Ser-globulin.	Ser-albumin.	Lecithin, Fat, Salts, etc.	Ser-globulin / Ser-albumin.	Authority.
Man	92.07	76.20	31.04	45.16	15.88	$\frac{1}{1.5}$	HAMMARSTEN[2]
Horse	85.97	72.57	45.65	26.92	13.40	$\frac{1}{0.591}$	"
Ox	89.65	74.99	41.69	33.30	14.66	$\frac{1}{0.842}$	"
Dog	.....	58.20	20.50	37.70	.....	$\frac{1}{1.8}$	SALVIOLI[3]
Hen	54.00	39.49	7.84	31.65	14.51	$\frac{1}{4.03}$	HAMMARSTEN
Frog	.....	25.40	21.80	3.60	.....	$\frac{1}{0.165}$	HALLIBURTON[4]
Eel	.....	67.30	52.80	14.50	.....	$\frac{1}{0.275}$	"

According to HALLIBURTON, the amount of the albumins in comparison with the globulins in cold-blooded animals is not only proportionally smaller, but the total amount of albuminous bodies is smaller than in the warm-blooded animals.

By a comparative investigation of serum and plasma from the same individual, we find more serglobulin in the one than in the other. The reason for this may lie partly in the fact that in the coagulation of fibrin from the fibrinogen some fibrin-globulin is formed which in the quantitative estimation is precipitated with the serglobulin, and partly because the white corpuscles yield serglobulin in the fibrin coagulation (ALEX. SCHMIDT).

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[1] Pflüger's Archiv, Bd. 17.

[2] *Ibid.*

[3] Du Bois-Reymond's Archiv, 1881, S. 275.

[4] Journ. of Physiol., Vol. 7, pp. 319–331.

The quantity of mineral bodies in the serum has been determined by many investigators.

The conclusions drawn from the analyses is that there exists a rather close correspondence between human and animal blood-serum, and it is therefore sufficient to give here the analysis of C. SCHMIDT[1] of (1) human blood, and of (2) pig- and (3) ox-blood by BUNGE.[2] As in the calcination of lecithin and proteids incorrect results are obtained for the phosphoric and sulphuric acids, these results will not be given below. All figures correspond to 1000 parts of serum.

	1	2	3
K_2O..........	0.387–0.401	0.273	0.254
Na_2O..........	4.290–4.290	4.272	4.351
Cl.......	3.565–3.659	3.611	3.717
CaO..........	0.155–0.155	0.136	0.126
MgO..........	0.101......	0.038	0.045
Fe_2O_3..........	....	0.011	0.011

The amount of NaCl is about 6 p. m., and it is remarkable that this amount of NaCl remains almost constant, so that with food containing an excess of NaCl it is quickly eliminated by the urine, and with food poor in chlorides the amount in the blood first decreases, but increases after taking chlorides from the tissues. The secretion of chlorides by the urine is thereby diminished.

The amount of phosphoric acid, calculated as Na_2HPO_4, in the serum freed from lecithin has been determined as 0.02–0.09 p. m. by SERTOLI[3] and MROCZKOWSKI[4] in different varieties of serum. The small amount of iron sometimes found in the serum probably originates from a contamination with the blood-coloring matters.

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[1] Cit. Hoppe-Seyler's Physiol. Chem., 1881, S. 439.

[2] Zeitschr. f. Biologie, Bd. 12, S. 206–208.

[3] Hoppe-Seyler's Med. chem. Untersuch., S. 350.

[4] Centralbl. f. d. med. Wissensch., 1878, No. 20.

II. The Form-elements of the Blood.

The Red Blood-corpuscles.

The blood-corpuscles are round, biconcave disks without membrane and nucleus in man and mammalia (with the exception of the llama, the camel, and their congeners). In the latter animals, as also in birds, amphibia, and fishes (with the exception of the cyclostoma), the corpuscles have in general a nucleus, are biconvex and more or less elliptical. The size varies in different animals. In man they have an average diameter of 7 to 8 μ ($\mu = 0.001$ mm.) and a maximum thickness of 1.9μ. The volume of a single red corpuscle of a horse amounts to 0.00000003858 c.mm. and of a pig 0.0000000435 c.mm. (WENDELSTADT and L. BLEIBTREU[1]). The weight of a red corpuscle of a horse is, according to the same investigators, 0.00000004307 mg. Their specific gravity is 1.088 to 1.105. They are heavier than the blood-plasma or serum, and therefore sink in these liquids. In the discharged blood they may lie sometimes with their flat surfaces together, forming a cylinder like a roll of coin. The reason for this is unknown, but as it may be observed in defibrinated blood it seems probable that the formation of fibrin has nothing to do with it. Seen with the microscope, each blood-corpuscle has a pale yellow color, and only in moderately thick layers is the color somewhat reddish.

The number of red blood-corpuscles is different in the blood of various animals. In the blood of man there are generally 5 million red corpuscles in 1 c.mm., and in woman 4 to 4.5 million.

On diluting the blood with water and alternately freezing and thawing it, as also on shaking it with ether, or by the action of chloroform or bile, a remarkable change takes place. The blood-coloring matters, which are hardly free in the blood-corpuscles, but rather, according to the view of HOPPE-SEYLER, are combined with some other substance, perhaps lecithin, are by this means set free from these combinations and pass into solution, while the remainder of each blood-corpuscle forms a swollen mass. By the action of carbon dioxide, by the careful addition of acids, acid salts, tincture of iodine, or certain other bodies, this residue, rich in albumin, condenses and in many cases the form of the blood-corpuscles may be again obtained. This residue has been called the *stroma* of the red blood-corpuscles.

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[1] Pflüger's Archiv, Bd. 52.

To isolate the stromata of the blood-corpuscles they are washed first by diluting the blood with 10–20 vols. of a 1–2% common-salt solution and then separating the mixture by centrifugal force or by allowing it to stand at a low temperature. This is repeated a few times until the blood-corpuscles are freed from serum. These purified blood-corpuscles are, according to WOOLDRIDGE, mixed with 5–6 vols. of water and then a little ether is added until complete solution is obtained. The leucocytes gradually settle to the bottom, a movement which may be accelerated by centrifugal force, and the liquid which separates therefrom is very carefully treated with a 1% solution of $KHSO_4$ until it is about as dense as the original blood. The separated stromata are collected on a filter and quickly washed.

WOOLDRIDGE[1] found as constituents of the stroma *lecithin*, *cholesterin*, *nucleoalbumin*, and a *globulin* which, according to HALLIBURTON, is probably a nucleoproteid which he calls *cell-globulin*. No nuclein substances or seralbumin or albumoses could be detected by HALLIBURTON and FRIEND.[2] The nucleated red blood-corpuscles of the bird contain, according to PLÓSZ and HOPPE-SEYLER,[3] *nuclein* and an albuminous body which swells to a slimy mass in a 10% common-salt solution, and which seems to be closely related to the hyaline substance (*hyaline substance* of ROVIDA) occurring in the lymph-cells. The red blood-corpuscles without any nucleus are, as a rule, very poor in proteid but are rich in hæmoglobin; the nucleated corpuscles are richer in proteid and poorer in hæmoglobin.

A gelatinous, fibrin-like proteid body may be obtained from the red blood-corpuscles under certain circumstances. This fibrin-like mass has been observed on freezing and then thawing the sediment of the blood-corpuscles, or on discharging the spark from a large Leyden jar through the blood, or on dissolving the blood-corpuscles of one kind of animal in the serum of another (LANDOIS, *stroma-fibrin*[4]). In none of these cases has it been shown that we have to deal with a fibrin formation at the expense of the stroma. It seems only to have been shown that the red blood-corpuscles of frog's blood contain fibrinogen (ALEX. SCHMIDT and SEMMER[1]).

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[1] Du Bois-Reymond's Archiv, 1881, S. 387.

[2] Journal of Physiol., Vol. 10.

[3] Hoppe-Seyler's Med. chem. Untersuch., S. 510.

[4] Centralbl. f. d. med. Wissensch., 1874, S. 421.

The *mineral bodies* of the red corpuscles are chiefly potassium, phosphoric acid, and chlorine; in the red corpuscles of man, the dog, and the ox sodium has also been found.

The most important constituent of the blood-corpuscles from a physiological standpoint is the red coloring matter.

Blood-coloring Matters.

According to HOPPE-SEYLER[2] the coloring matter of the red blood-corpuscles is not in a free state, but combined with some other substance. The crystalline coloring matter, the hæmoglobin or oxyhæmoglobin, which may be isolated from the blood, is considered, according to HOPPE-SEYLER, as a cleavage product of this combination, and it acts in many ways unlike the questionable combination itself. This combination is insoluble in water and uncrystallizable. It strongly decomposes hydrogen peroxide without being oxidized itself; it shows a greater resistance to certain chemical reagents (as potassium ferricyanide) than the free coloring matter, and lastly it gives off its loosely combined oxygen much more easily in vacuum than the free pigment. To distinguish between the cleavage products, the hæmoglobin and the oxyhæmoglobin, HOPPE-SEYLER[3] calls the combination of the blood-coloring matter of the venous blood-corpuscles *phlebin*, and that of the arterial *arterin*. Since the above-mentioned combination of the blood-coloring matters with other bodies, for example (if they really do exist) with lecithin, have not been closely studied, the following statements will only apply to the free pigment, the hæmoglobin.

The color of the blood depends in part on *hæmoglobin* or *pseudo-hæmoglobin* (see below), and in part on a molecular combination of this with oxygen, the *oxyhæmoglobin*. We find in blood after asphyxiation almost exclusively hæmoglobin and pseudo-hæmoglobin, in arterial blood disproportionately large amounts of oxyhæmoglobin, and in venous blood a mixture of both. Blood-coloring matters are found also in striated as well as in certain smooth muscles, and lastly in solution in different invertebrates. The quantity of hæmoglobin in human blood may indeed be somewhat variable under different circumstances, but amounts averaging about 14% or 8.5 grammes have been determined for each kilo of the weight of the body.

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[1] Alex. Schmidt in Pflüger's Archiv, Bd. 11, S. 550–559.

[2] Zeitschr. f. physiol. Chem., Bd. 13.

[3] *Ibid.*, S. 495.

Hæmoglobin belongs to the group of compound proteids, and yields as cleavage products, besides very small amounts of volatile fatty acids and other bodies, chiefly *proteid* (96%) and a coloring matter, *hæmochromogen* (4%), containing iron, which in the presence of oxygen is easily oxidized into *hæmatin*.

The hæmoglobin prepared from different kinds of blood has not exactly the same composition, which seems to indicate the presence of different hæmoglobins. The analyses of different investigators of the hæmoglobin from the same kind of blood do not always agree with one another, which probably depends upon the somewhat various methods of preparation. The following analyses are given as examples of the constitution of different hæmoglobins:

Hæmoglobin from the	C	H	N	S	Fe	O	P_2O_5	
Dog......	53.85	7.32	16.17	0.390	0.430	21.84	.....	(HOPPE-SEYLER[1])
"	54.57	7.22	16.38	0.568	0.336	20.93	.....	(JAQUET[2])
Horse	54.87	6.97	17.31	0.650	0.470	19.73	.....	(KOSSEL[3])
"	51.15	6.76	17.94	0.390	0.335	23.43	.....	(ZINOFFSKY[4])
Ox........	54.66	7.25	17.70	0.447	0.400	19.543	.....	(HUFNER[5])
Pig.......	54.17	7.38	16.23	0.660	0.430	21.360	.....	(OTTO[6])
"	54.71	7.38	17.43	0.479	0.399	19.602	.....	(HÜFNER)
Guinea-pig	54.12	7.36	16.78	0.580	0.480	20.680	.....	(HOPPE-SEYLER)
Squirrel ..	54.09	7.39	16.09	0.400	0.590	21.440	.....	"
Goose.....	54.26	7.10	16.21	0.540	0.430	20.690	0.77	"
Hen......	52.47	7.19	16.45	0.857	0.335	22.500	0.197	(JAQUET)

The question whether the amount of phosphorus in the hæmoglobin from birds exists as a contamination or as a constituent has not been decided. According to INOKO[7] the hæmoglobin from goose's blood consists of a combination between nucleic acid and hæmoglobin. In the hæmoglobin from the horse (ZINOFFSKY), the pig, and the ox (HÜFNER) we have 1 atom of iron to 2 atoms of sulphur, while in the hæmoglobin from the dog (JAQUET) the relation is 1 to 3. From the data of the elementary analysis, as

[1] Med. chem. Untersuch., S. 370.

[2] Zeitschr. f. physiol. Chem., Bd. 14, S. 296.

[3] *Ibid.*, Bd. 2, S. 150.

[4] *Ibid.*, Bd. 10.

[5] Beitr. z. Physiol., Festschr. f. C. Ludwig, 1887, S. 74–81.

[6] Zeitschr. f. physiol. Chem., Bd. 7, S. 61.

[7] *Ibid.*, Bd. 18.

also from the amount of loosely combined oxygen, HÜFNER[1] has calculated the molecular weight of dog-hæmoglobin as 14,129 and the formula $C_{636}H_{1025}N_{164}FeS_3O_{181}$. The molecular weight is therefore very high. The hæmoglobin from various kinds of blood not only shows a diverse constitution, but also a different solubility and crystalline form, and a varying quantity of water of crystallization; hence we infer that there are several kinds of hæmoglobin.

BOHR[2] is a very zealous advocate of this supposition. He has been able to obtain hæmoglobin from dog and horse blood, by fractional crystallization, which had different power of combining with oxygen and containing different quantities of iron. HOPPE-SEYLER[3] had already prepared two different forms of hæmoglobin crystals from horse's blood, and BOHR concludes from a collection of these observations that the ordinary hæmoglobin consists of a mixture of different hæmoglobins. In opposition to this statement HÜFNER[4] has shown that only one hæmoglobin exists in ox blood, and that this is probably true for the blood of many other animals.

Oxyhæmoglobin, which has also been called HÆMATOGLOBULIN or HÆMATOCRYSTALLIN, is a molecular combination of hæmoglobin and oxygen. For each molecule of hæmoglobin 1 molecule of oxygen exists; and the amount of loosely combined oxygen which is united to 1 grm. hæmoglobin (of the dog) has been determined by HÜFNER as 1.34 c.c. (calculated at 0° C. and 760 mm. mercury).

According to BOHR[2] the facts are different. He differentiates between four different oxyhæmoglobins, according to the quantity of oxygen which they absorb, namely, [illegible] and δ-oxyhæmoglobin, all having the same absorption-spectrum, and 1 gm. combining with respectively [illegible] and 2.7 c.c. oxygen at the temperature of the room and with an oxygen pressure of 150 mm. mercury. [illegible] the crystalline [illegible] is the quantity of iron is increased [illegible]

[1] [illegible] Chem. Bd. 22.

[2] Sur les [illegible] de l'hémoglobine avec l'oxygène. Extrait du [illegible] Royale Danoise des sciences 189[illegible]. Also Centralbl. f. Physiol. 189[illegible], S. [illegible]

[3] [illegible] Chem. Bd. 2.

[4] [illegible]-Reymond's Archiv.

γ-oxyhæmoglobin in a sealed tube it is transformed into δ-oxyhæmoglobin, although the circumstances of this change are not known. According to Hüfner[1] these are nothing but a mixture of genuine and partly decomposed hæmoglobins.

The ability of hæmoglobin to take up oxygen seems to be a function of the iron it contains, and when this is calculated as about 0.33–0.40%, then 1 atom of iron in the hæmoglobin corresponds to about 2 atoms = 1 molecule of oxygen. The combination of hæmoglobin with oxygen is, as has been stated, loose and dissociatable, and the quantity of oxygen taken up by a hæmoglobin solution depends upon the pressure of the oxygen at that temperature. If this latter be decreased by means of a vacuum, especially on gently heating or by passing some indifferent gas through the solution, all of the oxygen may be expelled from an oxyhæmoglobin solution so that only hæmoglobin remains. The reverse of this is true of a hæmoglobin solution which by its remarkable attraction for oxygen may be converted into oxyhæmoglobin. Oxyhæmoglobin is generally considered as a weak acid.

Oxyhæmoglobin has been obtained in crystals from several varieties of blood. These crystals are blood-red, transparent, silky, and may be 2–3 mm. long. The oxyhæmoglobin from squirrel's blood crystallizes in six-sided plates of the hexagonal system; the other varieties of blood yield needles, prisms, tetrahedra, or plates which belong to the rhombic system. The quantity of water of crystallization varies between 3–10% for the different oxyhæmoglobins. When completely dried at a low temperature over sulphuric acid the crystals may be heated to 110–115° C. without decomposing. At higher temperatures, somewhat above 160° C., they decompose, giving an odor of burnt horn, and leave, after complete combustion, an ash consisting of oxide of iron. The oxyhæmoglobin crystals from difficultly crystallizable kinds of blood, for example from such as ox's, human, and pig's blood, are easily soluble in water. The oxyhæmoglobin from easily crystallizable blood, as from that of the horse, dog, squirrel, and guinea-pig, are soluble with difficulty in the order above given. The oxyhæmoglobin dissolves more easily in a very dilute solution of alkali carbonate than in pure water, and this solution may be kept. The presence of a little too much alkali causes the oxyhæmoglobin to quickly decompose. The crystals are insoluble without decolorization in absolute alcohol. According to NENCKI,[1] it is hereby converted into an isomeric or polymeric modification, called by him *parahæmoglobin*. Oxyhæmoglobin is insoluble in ether, chloroform, benzol, and carbon disulphide.

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[1] Du Bois-Reymond's Archiv, 1894.

A solution of oxyhæmoglobin in water is precipitated by many metallic salts, but is not precipitated by sugar of lead or basic lead acetate. On heating the watery solution it decomposes at 60° to 70° C., and it splits off proteid and hæmatin. It is also decomposed by acids, alkalies, and many metallic salts. It gives the ordinary reactions for proteids with the ordinary proteid reagents which first decompose the oxyhæmoglobin with the splitting off of proteid.

The oxyhæmoglobin may, when it is gradually oxidized, act as an "ozone exciter" by the decomposition of neutral oxygen, converting it into active oxygen (PFLÜGER[2]). It may also have another relation to ozone, since it has the property of an "ozone transmitter" in that it causes the reaction of certain reagents (turpentine) containing ozone upon ozone reagents such as tincture of guaiacum.

A sufficiently dilute solution of oxyhæmoglobin or arterial blood shows a spectrum with two absorption-bands between the FRAUNHOFER lines *D* and *E*. The one band, α, which is narrower but darker and sharper, lies on the line *D*; the other, broader, less defined and less dark band, β, lies at *E*. These bands can be detected in a layer of 1 cm. thick of a 0.1 p. m. solution of oxyhæmoglobin. In a still weaker dilution the band β first disappears. By increased concentration of the solution the two bands become broader, the space between them smaller or entirely obliterated, and at the same time the blue and violet part of the spectrum is darkened. The oxyhæmoglobin may be differentiated from other coloring matters having a similar absorption-spectrum by its behavior towards reducing substances. (See below.)

A great many methods have been proposed for the preparation of oxyhæmoglobin crystals, but in their chief features they all agree with the following method as suggested by HOPPE-SEYLER[3]: The washed blood-corpuscles (best those from the dog or the horse) are stirred with 2 vols. water and then shaken with ether. After decanting the ether and allowing the ether which is retained by the blood solution to evaporate in an open dish in the air, cool the filtered blood solution to 0° C., add while stirring ¼ vol. of alcohol also cooled, and allow to stand a few days at − 5° to − 10° C. The crystals which separate may be repeatedly recrystallized by dissolving in water of about 35° C., cooling and adding cooled alcohol as above. Lastly, they are washed with cooled water containing alcohol (¼ vol. alcohol) and dried in vacuum at 0° C. or a lower temperature. According to GSCHEIDLEN's[1] investigations, oxyhæmoglobin crystals may be obtained from difficultly crystallizable varieties of blood by allowing the blood first to putrefy slightly in sealed tubes. After shaking with air by which the blood is again arterialized, proceed as above.

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[1] Nenchi and Sieber, Ber. d. deutsch. chem. Gesellsch., Bd. 18.

[2] Pflüger's Archiv, Bd. 10, S. 252.

[3] Med. chem. Untersuch., S. 181.

For the preparation of oxyhæmoglobin crystals in small quantities from blood easily crystallized, it is often sufficient to stir a drop of blood with a little water on a microscope slide and allow the mixture to evaporate so that the drop is surrounded by a dried ring. After covering with a thin glass, the crystals gradually appear radiating from the ring. These crystals are formed in a surer manner if the blood is first mixed with some water in a test-tube and shaken with ether and a drop of the lower deep-colored liquid treated as above on the slide.

Hæmoglobin, also called REDUCED HÆMOGLOBIN or PURPLE CRUORIN (STOKES[2]), occurs only in very small quantities in arterial blood, in larger quantities in venous blood, and is nearly the only blood-coloring matter after asphyxiation.

Hæmoglobin is much more soluble than the oxyhæmoglobin, and it can therefore only be obtained as crystals with difficulty. These crystals are as a rule isomorphous to the corresponding oxyhæmoglobin crystals, but are darker, having a shade towards blue or purple, and are decidedly more pleochromatic. Its solutions in water are darker and more violet or purplish than solutions of oxyhæmoglobin of the same concentration. They absorb the blue and the violet rays of the spectrum in a less marked degree, but strongly absorb the rays lying between *C* and *D*. In proper dilution the solution shows a spectrum with one broad, not sharply defined band between *D* and *E*. This band does not lie in the middle between *D* and *E*, but is towards the red end of the spectrum, a little over the line *D*. A hæmoglobin solution actively absorbs oxygen from the air and is converted into an oxyhæmoglobin solution.

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[1] Pflüger's Archiv, Bd 16.

[2] Philosophical Magazine, Vol. 28, No. 190, Nov. 1864.

A solution of oxyhæmoglobin may be easily converted into a solution having the spectrum of hæmoglobin by means of a vacuum, by passing an indifferent gas through it, or by the addition of a reducing substance, as, for example, an ammoniacal ferro-tartarte solution (STOKES' reduction-liquid). If an oxyhæmoglobin solution or arterial blood is kept in a sealed tube, we observe a gradual consumption of oxygen and a reduction of the oxyhæmoglobin into hæmoglobin. If the solution has a proper concentration, a crystallization of hæmoglobin may occur in the tube at lower temperatures (HÜFNER[1]).

Pseudohæmoglobin. LUDWIG and SIEGFRIED[2] have observed that blood which has been reduced by hyposulphites so completely that the oxyhæmoglobin spectrum disappears and only the hæmoglobin spectrum is seen yields large amounts of oxygen when exposed to a vacuum. Blood which has been reduced by the passage of a stream of hydrogen through it until the oxyhæmoglobin spectrum disappears acts in the same manner. Hence a loose combination of hæmoglobin and oxygen exists which gives the hæmoglobin spectrum, and this combination is called pseudohæmoglobin by LUDWIG and SIEGFRIED. Pseudohæmoglobin, whose presence has been detected in asphyxiation blood from dogs, is considered by the AUTHOR as an intermediate step between hæmoglobin and oxyhæmoglobin, on the reduction of the latter.

Methæmoglobin. This name has been given to a coloring matter which is easily obtained from oxyhæmoglobin as a transformation product and which has been correspondingly found in transudations and cystic fluids containing blood, in urine, in hæmaturia or hæmoglobinuria, also in urine and blood on poisoning with potassium chlorate, amyl nitrite or alkali nitrite, and many other bodies.

Methæmoglobin does not contain any oxygen in molecular or dissociable combination, but still the oxygen seems to be of importance in the formation of methæmoglobin, because it is formed from oxyhæmoglobin in the absence of oxygen or oxidizing agents, and not from hæmoglobin. If arterial blood be sealed up in a tube, it gradually consumes its oxygen and becomes venous, and by this absorption of oxygen a little methæmoglobin is formed. The same occurs on the addition of a small quantity of acid to the blood.

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[1] Zeitschr. f. physiol. Chem.. Bd. 4, S. 382.

[2] Du Bois-Reymond's Archiv, 1890; also see Ivo Novi, Pflüger's Archiv, Bd. 56.

By the spontaneous decomposition of blood some methæmoglobin is formed, and by the action of ozone, potassium permanganate, potassium ferricyanide, chlorates, nitrites, nitrobenzol, pyrogallol, pyrocatechin, acetanilid, and certain other bodies on the blood an abundant formation of methæmoglobin takes place.

According to the investigations of HÜFNER, KÜLZ, and OTTO[1] methæmoglobin contains just as much oxygen as oxyhæmoglobin, but it is more strongly combined. JÄDERHOLM[2] and SAARBACH[3] claim that a methæmoglobin solution is first converted into an oxyhæmoglobin and then into a hæmoglobin solution by reducing substances, while HOPPE-SEYLER and ARAKI[4] claim that it is converted directly into a hæmoglobin solution.

Methæmoglobin has the same constitution as oxyhæmoglobin (HÜFNER and OTTO). It was first shown by them that it crystallizes in brownish-red needles, prisms, or six-sided plates. It dissolves easily in water; the solution has a brown color and becomes a beautiful red on the addition of alkali. The solution of the pure substance is not precipitated by basic lead acetate alone, but by basic lead acetate and ammonia. The absorption-spectrum of a watery or acidified solution of methæmoglobin is, according to JÄDERHOLM and BERTIN-SANS,[5] very similar to that of hæmatin in acid solution, but is easily distinguished from the latter since, on the addition of a little alkali and a reducing substance, the former passes over to the spectrum of reduced hæmoglobin, while a hæmatin solution under the same conditions gives the spectrum of an alkaline hæmochromogen solution (see below). Methæmoglobin in alkaline solution shows two absorption-bands which are like the two oxyhæmoglobin bands, but they differ from these in that the band β is stronger than α. By the side of the band α and united with it by a shadow lies a third, fainter band between C and D, near to D. According to other investigators, ARAKI and DITTRICH,[6] a neutral or faintly acid methæmoglobin solution shows only one characteristic band α between C and D, and the second band between *D* and *E* is only due to contamination with oxyhæmoglobin.

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[1] Zeitschr. f. physiol. Chem., Bd. 7.

[2] Nord. med. Arkiv, Bd. 16, and Zeitschr. f. Biologie, Bd. 16.

[3] Pflüger's Archiv, Bd. 28.

[4] Zeitschr. f. physiol. Chem., Bd. 14.

[5] Compt. rend., Tome 106.

[6] Arch. f. exp. Path. u. Pharm., Bd. 29. Important references on methæmoglobin are given by Otto, Pflüger's Archiv, Bd. 31.

Crystallized methæmoglobin may be easily obtained by treating a concentrated solution of oxyhæmoglobin with a sufficient quantity of concentrated potassium ferricyanide solution to give the mixture a porter-brown color. After cooling to 0° C. add ¼ vol. cooled alcohol and allow the mixture to stand a few days in the cold. The crystals may be easily purified by recrystallizing from water by the addition of alcohol.

Carbon Monoxide Hæmoglobin[1] is the molecular combination between 1 mol. hæmoglobin and 1 mol. CO. This combination is stronger than the oxygen combination of hæmoglobin. The oxygen is for this reason easily driven off by carbon monoxide, and this explains the poisonous action of carbon monoxide, which kills by the expulsion of the oxygen of the blood. HÜFNER[2] has determined the dissociation constant of carbon-monoxide hæmoglobin and finds it equal to 0.074 for a solution containing on an average 11 gm. in 100 c.c. at a temperature of 32.7° C. The dissociation constant of carbon monoxide hæmoglobin is hence about 33 times smaller than that of oxyhæmoglobin under nearly the same conditions (K for oxyhæmoglobin $= 2.44$).

Carbon monoxide hæmoglobin is formed by saturating blood or a hæmoglobin solution with carbon monoxide, and may be obtained as crystals by the same means as oxyhæmoglobin. These crystals are isomorphous to the oxyhæmoglobin crystals, but are less soluble and more stable, and their bluish-red color is more marked. For the detection of carbon-monoxide hæmoglobin its absorption spectrum is of the greatest importance. This spectrum shows two bands which are very similar to those of oxyhæmoglobin, but they occur more towards the violet part of the spectrum. These bands do not change noticeably on the addition of reducing substances; this constitutes an important difference between carbon monoxide and oxyhæmoglobin. If the blood contains oxyhæmoglobin and carbon-monoxide hæmoglobin at the same time, we obtain on the addition of a reducing substance (ammoniacal ferrotartrate solution) a mixed spectrum originating from the hæmoglobin and carbon-monoxide hæmoglobin.

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[1] In reference to carbon monoxide hæmoglobin see especially Hoppe-Seyler, Med. chem. Untersuch., S. 201; Centralbl. f. d. med. Wissensch., 1864 and 1865; Zeitschr. f. physiol. Chem., Bdd. 1 and 13.

[2] Du Bois-Reymond's Archiv, Physiol. Abth., 1895.

A great many reactions have been suggested for the detection of carbon-monoxide hæmoglobin in medico-legal cases. A simple and at the same time a good one is HOPPE-SEYLER's soda test. The blood is treated with double its volume of caustic-soda solution of 1.3 sp. gr., by which ordinary blood is converted into a dingy brownish mass, which when spread out on porcelain is brown with a shade of green. Carbon-monoxide blood gives under the same conditions a red mass, which if spread out on porcelain shows a beautiful red color. Several modifications of this test have been proposed.

Carbon monoxide methæmoglobin has been prepared by WEIL and v. ANREP[1] by the action of potassium permanganate on carbon monoxide hæmoglobin, but this is contradicted by BERTIN-SANS and MOITESSIER.[2] **Sulphur methæmoglobin** is the name given by HOPPE-SEYLER[3] to that coloring matter which is formed by the action of sulphuretted hydrogen on oxyhæmoglobin. The solution has a greenish-red, dirty color and shows an absorption-band in the red. This coloring matter is claimed to be the greenish color seen on the surface of putrefying flesh.

Carbon-dioxide Hæmoglobin, *Carbohæmoglobin.* Hæmoglobin, according to BOHR[4] and TORUP,[5] also forms a molecular combination with carbon dioxide whose spectrum is similar to that of hæmoglobin. According to BOHR there are three different carbohæmoglobins, namely, α-, β-, and γ-carbohæmoglobin, in which 1 gm. combines with respectively 1.5, 3, and 6 c.c. CO_2 (measured at 0° C. and 760 mm.) at + 18° C. and a pressure of 60 mm. mercury. If a hæmoglobin solution is shaken with a mixture of oxygen and carbon dioxide, the hæmoglobin combines loosely with the oxygen as well as carbon dioxide, independently of each other, just as if each gas existed alone (BOHR). He considers that the two gases are combined with different parts of the hæmoglobin, namely, the oxygen with the pigment nucleus and the carbon dioxide with the proteid component. According to TORUP the hæmoglobin must therefore be partly decomposed by the carbon dioxide setting free some proteid.

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[1] Du Bois-Reymond's Archiv, 1880.

[2] Compt. rend., Tome 113.

[3] Med. chem. Untersuch., S. 151; also see Araki, Zeitschr. f. physiol. Chem., Bd. 14.

[4] "Etudes sur les combinaisons du sang avec l'acide carbonique." Extrait du Bull. de l'Acad. Danoise, 1890, also Centralbl. f. Physiol., Bd. 4, 1890.

[5] Maly's Jahresber., Bd. 17, S. 115.

Nitric-oxide Hæmoglobin[1] is also a crystalline molecular combination which is even stronger than the carbon-monoxide hæmoglobin. Its solution shows two absorption-bands which are paler and less sharp than the carbon-monoxide hæmoglobin bands, and they do not disappear on the addition of reducing bodies.

Hæmoglobin also forms a molecular combination with *acetylene*. Methæmoglobin solutions become of a beautiful red color by the action of hydrocyanic acid, and, according to KOBERT,[2] *cyanmethæmoglobin* is probably formed. Its spectrum is very similar to that of hæmoglobin, but it is not converted into oxyhæmoglobin on shaking with air.

Decomposition products of the blood-coloring matters. By its decomposition hæmoglobin yields, as above stated, a *proteid*, which has been called *globin*, and a ferruginous *pigment* as chief products. If the decomposition takes place in the absence of oxygen, a coloring matter is obtained which is called by HOPPE-SEYLER *hæmochromogen*, by other investigators (STOKES) *reduced hæmatin*. In the presence of oxygen, hæmochromogen is quickly oxidized to hæmatin, and we therefore obtain in this case *hæmatin* as a colored decomposition product. As hæmochromogen is easily converted by oxygen into hæmatin, so this latter may be reconverted into hæmochromogen by reducing substances.

Hæmochromogen was discovered by HOPPE-SEYLER.[3] He has also been able to obtain this coloring matter as crystals. Hæmochromogen is, according to HOPPE-SEYLER, the colored atomic group of hæmoglobin and its combination with gases, and this atomic group is combined with proteids in the coloring matter. The characteristic absorption of light depends on the hæmochromogen, and it is also this atomic group which binds in the oxyhæmoglobin 1 mol. oxygen and in the carbon-monoxide hæmoglobin 1 mol. carbon monoxide with 1 atom iron. HOPPE-SEYLER has observed a combination between hæmochromogen and carbon monoxide, and this combination shows the spectral appearance of carbon monoxide hæmoglobin.

An alkaline hæmochromogen solution has a beautiful red color. It shows two absorption-bands, first described by STOKES, of which the one is darker and lies between D and E, and the other, broader but not so dark, covers the lines *E* and *b*. In acid solution hæmochromogen shows four bands, which, according to JÄDERHOLM,[1] depend on a mixture of hæmochromogen and hæmatoporphyrin (see below), this last formed by a partial decomposition resulting from the action of the acid.

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[1] See Herrmann and Reichert in Du Bois-Reymond's Archiv, 1865, and Hoppe-Seyler, Med. chem. Untersuch., S. 204.

[2] Ueber Cyanmethæmoglobin und den Nachweis der Blausäure. Stutt[illegible] 1891.

[illegible]schr. f. physiol. Chem., Bd. 12.

Hæmochromogen may be obtained as crystals by the action of caustic soda on hæmoglobin at 100° C. in the absence of oxygen (HOPPE-SEYLER). By the decomposition of hæmoglobin by acids (of course in the absence of air) we obtain hæmochromogen contaminated with a little hæmatoporphyrin. An alkaline hæmochromogen solution is easily obtained by the action of a reducing substance (STOKES' reduction liquid) on an alkaline hæmatin solution.

Hæmatin, also called **Oxyhæmatin**, is sometimes found in old transudations. It is formed by the action of gastric or pancreatic juices on oxyhæmoglobin, and is therefore also found in the fæces after hemorrhage in the intestinal canal, and also after a meat diet and food rich in blood. It is stated that hæmatin may occur in urine after poisoning with arseniuretted hydrogen. As shown above, the hæmatin is formed by the decomposition of oxyhæmoglobin, or at least of hæmoglobin, in the presence of oxygen. BERTIN-SANS and MOITESSIER[2] have prepared an intermediate body between oxyhæmoglobin and hæmochromogen. This reduced hæmatin shows one band whose middle lies over *D*.

The constitution of hæmatin may, according to HOPPE-SEYLER,[3] be expressed by the formula $C_{34}H_{35}N_4FeO_5$. According to NENCKI and SIEBER it has the formula $C_{32}H_{32}N_4FeO_4$, and they claim that hæmatin is a hydrate of a body not yet isolated, hæmin, $C_{32}H_{30}N_4FeO_3$.

Hæmatin is amorphous, dark brown or bluish black. It may be heated to 180° C. without decomposition; on burning it leaves a residue consisting of iron oxide. It is insoluble in water, dilute acids, alcohol, ether, and chloroform, but it dissolves slightly in warm glacial acetic acid. Hæmatin dissolves in acidified alcohol or ether. It easily dissolves in alkalies, even when very dilute. The alkaline solutions are dichroitic; in thick layers they appear red by transmitted light, and in thin layers greenish. The alkaline solutions are precipitated by lime- and baryta-water, as also by solutions of neutral salts of the alkaline earths. The acid solutions are always brown.

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[1] Nord. med. Arkiv, Bd. 16.

[2] Compt. rend., Tome 116.

[3] Med. chem. Untersuch., S. 525.

An acid hæmatin solution absorbs the red part of the spectrum less and the violet part more. The solution shows a rather sharply defined band between *C* and *D* whose position may change with the variety of acid used as a solvent. Between *D* and *F* a second, much broader, less sharply defined band occurs which by proper dilution of the liquid is converted into two bands. The one between *b* and *F*, lying near *F*, is darker and broader, the other, between *D* and *E*, lying near *E*, is lighter and narrower. Also by proper dilution a fourth very faint band is observed between *D* and *E* lying near *D*. Hæmatin may thus in acid solution show four absorption-bands; ordinarily one sees distinctly only the bands between *C* and *D* and the broad, dark band—or the two bands—between *D* and *F*. In alkaline solution the hæmatin shows a broad absorption-band, which lies in greatest part between *C* and *D*, but reaches a little over the line *D* towards the right in the space between *D* and *E*.

Hæmin, HÆMIN CRYSTALS, or TEICHMANN'S CRYSTALS. Hæmin, according to HOPPE-SEYLER, is a combination between hæmatin and hydrochloric acid, having the formula $C_{34}H_{35}N_4FeO_5.HCl$. NENCKI and SIEBER designate as hæmin, on the contrary (see page 141), a body not yet isolated, of the formula $C_{32}H_{30}N_4FeO_3$, which may be considered as an anhydride of hæmatin or $C_{32}H_{32}N_4FeO_4 - H_2O$. The hæmin crystals are, according to the latest views, a combination of this substance, hæmin, and HCl, according to the formula $C_{32}H_{30}N_4FeO_3.HCl$. The analyses of the hydrochloride and hydrobromide of hæmatin by HÜFNER and KÜSTER[1] lead to the same formula.

According to NENCKI and SIEBER the hæmin crystals are a double combination with the solvent, amyl alcohol or acetic acid, which is used in their preparation; while HOPPE-SEYLER claims that the solvent is only held mechanically by the crystals. The formula of the hæmin crystals prepared by means of amyl alcohol is, according to NENCKI and SIEBER,

$$(C_{32}H_{30}N_4FeO_3.HCl)_4.C_5H_{12}O.$$

Hæmin crystals form in large masses a bluish-black powder, but are so small that they can only be seen by the microscope. They consist of dark-brown or nearly brownish-black, long, rhombic, or spool-like crystals, isolated, or grouped as crosses, rosettes, or starry forms. They are insoluble in water, dilute acids at the normal temperature, alcohol, ether, and chloroform. They are slightly dissolved by glacial acetic acid and warmth. They dissolve in acidified alcohol, as also in dilute caustic or carbonated alkalies; and in the last case they form, besides alkali chlorides, soluble hæmatin alkali, from which the hæmatin may be precipitated by an acid.

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[1] Ber. d. deutsch. chem. Gesellsch., Bd. 27, S. 572.

The preparation of hæmin crystals is always the starting-point for the preparation of hæmatin. According to HOPPE-SEYLER,[1] shake the blood-corpuscles which have been washed with common-salt solution with water and ether, then filter the solution of blood-coloring matters, concentrate strongly, mix with 10–20 vols. glacial acetic acid, and heat for 1–2 hours on the water-bath. After diluting with several volumes of water, allow the liquid to stand a few days. The crystals which separate are then washed with water, boiled with acetic acid, and then washed again with water, alcohol, and ether. NENCKI and SIEBER coagulate the sediment of the blood-corpuscles by alkali, allow the coagulum to dry incompletely in the air, rub it fine, and then boil it with amyl alcohol after the addition of a little hydrochloric acid. The crystals which separate from the filtrate after cooling are washed with water, alcohol, and ether. If hæmin crystals be dissolved in dilute caustic alkali, hæmatin may be precipitated from the solution by the addition of acid; and from this hæmatin pure hæmin crystals may be prepared by heating with glacial acetic acid and a little common salt.

In preparing hæmin crystals in small amounts proceed in the following manner: The blood is dried after the addition of a small quantity of common salt, or the dried blood may be rubbed with a trace of common salt. The dry powder is placed on a microscope-slide, moistened with glacial acetic acid, and then covered with the cover-glass. Add, by means of a glass rod, more glacial acetic acid by applying the drop at the edge of the cover-glass, until the space between the slide and the cover-glass is full. Now warm over a very small flame, with the precaution that the acetic acid does not boil and pass with the powder from under the cover-glass. If no crystals appear after the first warming and cooling, warm again, and if necessary add some more acetic acid. After cooling, if the experiment has been properly performed, a number of dark-brown or nearly black hæmin crystals of varying forms will be seen.

Hæmatin is dissolved by concentrated sulphuric acid in the presence of air, forming a purple-red liquid. The iron is here split off and the new coloring matter, called *hæmatoporphyrin* by HOPPE-

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[1] Med. chem. Untersuch., S. 379.

SEYLER,[1] is iron-free. The hæmatin yields with concentrated sulphuric acid, in the absence of air, a second iron-free coloring matter called *hæmatolin* (HOPPE-SEYLER). Hæmatoporphyrin may also be prepared by the action of glacial acetic acid saturated with hydrobromic acid on hæmin crystals (NENCKI and SIEBER[2]).

Hæmatoporphyrin, $C_{16}H_{18}N_2O_3$. This pigment, according to MAC MUNN,[3] occurs as a physiological coloring matter in certain animals. It has been repeatedly observed in the last few years in human urine especially after the use of sulphonal (see Chapter XV on the urine). This coloring matter is, according to NENCKI and SIEBER, an isomer of the bile-pigment bilirubin, and its formation from hæmatin can be expressed by the following equation:

$$C_{32}H_{32}N_4O_4Fe + 2H_2O - Fe = 2C_{16}H_{18}N_2O_3.$$

A pigment closely allied to the urinary pigment urobilin has been obtained by the action of reducing substances on hæmotoporphyrin (HOPPE-SEYLER,[4] NENCKI and SIEBER,[5] LE NOBEL,[6] MAC MUNN[7]). On the administration of hæmotoporphyrin to rabbits, NENCKI and ROTSCHY[8] observed that a part was reduced to a substance similar to urobilin.

The combinations of hæmotoporphyrin with Na and with HCl have been obtained as crystals by NENCKI and SIEBER. The acid alcoholic solutions have a beautiful purple color, which becomes violet-blue on the addition of large quantities of acid. The alkaline solution has a beautiful red color, especially when not too much alkali is present. Hæmatoporphyrin prepared by various methods may differ somewhat in solubility and in color of solution, but their characteristic absorption-spectra are essentially the same.

An alcoholic solution of hæmatoporphyrin, acidulated with hydrochloric or sulphuric acid, shows two absorption-bands, of which one is fainter and narrower and lies between *C* and *D*, near *D*. The other is much darker, sharper and broader and lies in the middle between *D* and *E*. An absorption extends from these bands towards the red, terminating with a dark edge, which may be considered as a third band between the other two.

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[1] Med. chem. Untersuch., S. 528.
[2] Monatshefte f. Chem., Bd. 9.
[3] Journ. of Physiol., Vol. 7.
[4] Med. chem. Untersuch., S. 533.
[5] Monatshefte f. Chem., Bd. 9.
[6] Pflüger's Archiv, Bd. 40.
[7] Proc. Roy. Soc., 1880, No. 208; Journ. of Physiol., Vol. 10.
[8] Monatshefte f. Chem., Bd. 10.

A dilute alkali solution shows four bands, namely, a band between *C* and *D*; a second, broader, surrounding *D* and with its broadest part between *D* and *E*; a third, between *D* and *E* nearly at *E*; and lastly a fourth, broad and dark band between *b* and *F*. On the addition of an alkaline zinc-chloride solution the spectrum changes more or less rapidly,[1] and finally a spectrum is obtained with only two bands, of which one surrounds *D* and the other lies between *D* and *E*.

Hæmatoidin, thus called by VIRCHOW, is a coloring matter which crystallizes in orange-colored rhombic plates, and which occurs in old blood extravasations, and whose origin from the blood-coloring matters seems to be established (LANGHANS, CORDUA, QUINCKE, and others[2]). A solution of hæmatoidin shows no absorption-bands, but only a strong absorption of the violet to the green (EWALD[3]). According to most observers, hæmatoidin is identical with the bile-coloring matter bilirubin. It is not identical with the crystallizable lutein from the *corpora lutea* of the ovaries of the cow (PICCOLO and LIEBEN,[4] KÜHNE and EWALD).

In the detection of the above-described blood-coloring matters the spectroscope is the only entirely trustworthy means of investigation. If it is only necessary to detect blood in general and not to determine definitely whether the coloring matter is hæmoglobin, methæmoglobin, or hæmatin, then the preparation of hæmin crystals is an absolute positive proof. The reader is referred to more extended text-books for exacter methods for the detection of blood in chemico-legal cases, and it is perhaps sufficient to give here the chief points of the investigation.

If spots on clothes, linen, wood, etc., are to be tested for the presence of blood, it is best, when possible, to scratch or shave off as much as possible, rub with common salt, and from this prepare the hæmin crystals. On obtaining positive results the presence of blood is not to be doubted. If you do not obtain sufficient material by the above means, then soak the spot with a few drops of water in a watch-crystal. If a colored solution is thus obtained, then remove the fibres, wood-shavings, and the like as far as possible, and allow the solution to dry in the watch-glass. The dried residue may be partly used for the spectroscope test directly, and part may be employed in the preparation of the hæmin crystals. It also serves to detect hæmochromogen in alkaline solution after previous treatment with alkali and the addition of reducing substances.

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[1] Hammarsten, Skan. Arch. f. Physiol., Bd. 3.

[2] A comprehensive review of the literature pertaining to hæmatoidin may be found in Stadelmann: Der Icterus, etc. Stuttgart, 1891. Pages 3 and 45.

[3] Zeitschr. f. Biologie, Bd. 22, S. 475.

[4] Cit. from Gorup-Besanez: Lehrbuch d. physiol. Chem., 4. Aufl., 1878.

If a colorless solution is obtained after soaking with water, or the spots are on rusty iron, then digest with a little dilute alkali (5 p. m.). In the presence of blood the solution gives, after neutralization with hydrochloric acid and drying, a residue which may give the hæmin crystals with glacial acetic acid. Another part of the alkaline solution shows, after the addition of STOKES' reduction liquid, the absorption-bands of hæmochromogen in alkaline solution.

The methods proposed for the quantitative estimation of the blood-coloring matters are partly chemical and partly physical.

Among the chemical methods to be mentioned is the ashing of the blood and the determination of the amount of iron contained therein, from which the amount of hæmoglobin may be calculated. Another method consists in first saturating the blood completely with oxygen. Now pump out thoroughly this oxygen, and calculate from the amount of oxygen the amount of hæmoglobin present (GRÉHANT[1] and QUINQUAUD[2]). None of these methods is reliable.

The physical methods consist either in a colorimetric or a spectroscopic investigation.

The principle of HOPPE-SEYLER'S *colorimetric method* is that a measured quantity of blood is diluted with an exactly measured quantity of water until the diluted blood solution has the same color as a pure oxyhæmoglobin solution of a known strength. The amount of coloring matter present in the undiluted blood may be easily calculated from the degree of dilution. In the colorimetric testing we use a glass vessel with parallel sides containing a layer of liquid 1 cm. thick (hæmatinometer of HOPPE-SEYLER). The method is good, and the inconvenience that the normal solution of oxyhæmoglobin does not keep for any length of time without decomposing may be prevented by preserving the solution in sealed tubes. The oxyhæmoglobin is gradually reduced to a hæmoglobin solution which may be kept for years, and when required for use it is converted into an oxyhæmoglobin solution by aerating. According to an improved method by HOPPE SEYLER,[3] it is much better to use a solution of carbon-monoxide hæmoglobin, as normal solution. The blood solution in this case is saturated with carbon monoxide and the two solutions compared in a specially constructed colorimetric double pipette (see original article). The replacing of the oxyhæmoglobin solution by a solution of picrocarmin, as suggested by certain investigators, is to be rejected according to HOPPE-SEYLER.

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[1] Compt. rend., Tome 75.

[2] *Ibid.*, Tome 76.

[3] Zeitschr. f. physiol. Chem., Bd. 16, and Lehrbuch d. physiol. u. pathol. chem. Analyse, 6. Aufl., 1893

The quantitative estimation of the blood-coloring matters by means of the spectroscope may be done in different ways, but at the present time the *spectrophotometric* method is chiefly used, and this seems to be the most reliable. This method[1] is based on the fact that the extinction coefficient of a colored liquid for a certain region of the spectrum is directly proportional to the concentration, so that $C : E = C_1 : E_1$, when C and C_1 represent the different concentrations and E and E_1 the corresponding coefficient of extinction. From the equation $\frac{C}{E} = \frac{C_1}{E_1}$ it follows that for one and the same coloring matter this relation, which is called the *absorption ratio*, must be constant. If the absorption ratio is represented by A, the determined extinction coefficient by E, and the concentration (the amount of coloring matter in grams in 1 c.c.) by C, then $C = A . E$.

Different apparatus have been constructed (VIERORDT and HÜFNER[2]) for the determination of the extinction coefficient which is equal to the negative logarithm of those rays of light which remain after the passage of the light through a layer 1 cm. thick of an absorbing liquid. In regard to these apparatus the reader is referred to other text-books.

As control the extinction coefficients are determined in two different regions of the spectrum, namely, $D32E—D54E$ and $D63E—D84E$. The constants or the absorption ratio for these two regions of the spectrum are designated by HÜFNER by A and A'. Before the determination the blood must be diluted with water, and if the proportion of dilution of the blood be represented by V, then the concentration or the amount of coloring matter in 100 parts of the undiluted blood is

$$C = 100 . V . A . E \text{ and}$$
$$C = 100 . V . A' \; E'.$$

The absorption ratio or the constants in the two above-mentioned regions of the spectrum have been determined for oxyhæmoglobin, hæmoglobin, carbon-monoxide hæmoglobin, and methæmoglobin.

The figures for the above coloring matters obtained from canine blood are as follows:

Oxyhæmoglobin..............	$A_o = 0.001330$	and	$A'_o = 0.001000$
Hæmoglobin.................	$A_r = 0.001091$	"	$A'_r = 0.001351$
Carbon-monoxide hæmoglobin	$A_c = 0.001130$	"	$A'_c = 0.001000$
Methæmoglobin.............	$A_m = 0.003696$	"	$A'_m = 0.002798$

The quantity of each coloring matter may be determined in a mixture of two blood-coloring matters by this method, which is of special importance in the determination of the quantity of oxyhæmoglobin and hæmoglobin present in blood at the same time. If we represent by E and E' the extinction coefficients of the mixture in the above-mentioned regions of the spectrum, by A_o and A'_o and A_r and A'_r the constants for oxyhæmoglobin and reduced hæmoglobin, and by V the degree of dilution of the blood, then the percentage of oxyhæmoglobin H_o and of (reduced) hæmoglobin H_r is

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[1] See Vierordt, Die Anwendung des Spektralapparates zu Photometrie, etc. (Tübingen, 1873), and Hüfner, Zeitschr. f. physiol. Chem., Bd. 3; v. Noorden, *Ibid.*, Bd. 4; Otto, *Ibid.*, Bd. 7; and Pflüger's Archiv, Bdd. 31 and 36.

[2] L. c.

$$H_o = 100 \,.\, V \,.\, \frac{A_o A'_o (E A_r - E' A'_r)}{A'_o A_r - A_o A'_r}$$

and

$$H_r = 100 \,.\, V \,.\, \frac{A_r A'_r (E' A'_o - E A_o)}{A'_o A_r - A_o A'_r}.$$

Among the many apparatus constructed for clinical purposes for the quantitative estimation of hæmoglobin the hæmometer of Fleischl[1] is to be preferred. The determination by this apparatus is made by comparing the color of the blood diluted with water with the color of a wedge-shaped movable prism of red glass. If the blood shows the same color as the glass prism, then the amount of hæmoglobin in the blood may be directly read from the scale. The amount of hæmoglobin is expressed as percentage of the physiological amount of hæmoglobin.

Many other coloring matters are found besides the often-occurring hæmoglobin in the blood of invertebra. In a few arachnidæ, crustacea, gasteropodæ, and cephalopodæ a body analogous to hæmoglobin containing copper, *hæmocyanin*, has been found by Fredericq.[2] By the taking up of loosely bound oxygen this body is converted into blue *oxyhæmocyanin*, and by the escape of the oxygen becomes colorless again. A coloring matter called *chlorocruorin* by Lankester[3] is found in certain chætopodæ. *Hæmerythrin*,[4] so called by Krukenberg but first observed by Schwalbe, is a red coloring matter from certain gephyrea. Besides hæmocyanin we find in the blood of certain crustacea the red coloring matter *tetronerythrin* (Halliburton[5]), which is also widely spread in the animal kingdom. *Echinochrom*, so named by Mac Munn,[6] is a brown coloring matter occurring in the perivisceral fluid of a variety of echinoderms.

The *quantitative constitution of the red blood-corpuscles* is difficult to determine, and we have hardly any sufficiently trustworthy analyses of them. The amount of water varies in different varieties of blood between 570–630 p. m., with a corresponding amount, 430–370 p. m., of solids. The chief mass, about $\frac{9}{10}$, of the dried substance consists of hæmoglobin (in human and canine blood).

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[1] See v. Jaksch, Klinische Diagnostik, 4. Aufl., p. 18.

[2] Extrait des Bulletins de l'Acad. Roy. de Belgique (2), Tome 46, 1878.

[3] Journ. of Anat. and Physiol., 1868, p. 114, and 1870, p. 119.

[4] See Physiol. Studien, Reihe 1, Abth. 3. Heidelberg, 1880.

[5] Journal of Physiol., Vol. 6.

[6] Quart. Journ. Microsc. Science, 1885.

According to the analyses of HOPPE-SEYLER[1] and his pupils, the red corpuscles contain in 1000 parts of the dried substance:

	Hæmoglobin.	Albumin.	Lecithin.	Cholesterin.
Human blood...	868–943	122–51	7.2–3.5	2.5
Dog "	865	126	5.9	3.6
Goose "	627	364	4.6	4.8
Snake "	467	525	...	...

Of special interest is the varying proportion of the hæmoglobin to the proteid in the nucleated and in the non-nucleated blood-corpuscles. These last are much richer in hæmoglobin and poorer in proteid than the others.

According to M. and L. BLEIBTREU and WENDELSTADT[2] the amount of nitrogen in the red corpuscles seems to be constant in certain animals, such as the horse and the pig. The quantity of proteid (inclusive of hæmoglobin) in the moist corpuscles of the horse was 468.5 and in the pig 443.6 p. m. as calculated by the above experiments from the quantity of nitrogen.

The amount of mineral bodies, as far as they have been determined, in the moist corpuscles is 4.8–8.9 p. m. The chief mass consists of potassium, phosphoric acid, and chlorine. The blood-corpuscles of ox-blood contain, according to BUNGE, more sodium and chlorine than phosphoric acid and potassium. The blood-corpuscles of the pig and horse contain no sodium (BUNGE[3]). Human-blood corpuscles contain, according to WANACH,[4] about five times as much potassium as sodium, on an average 3.99 p. m. potassium and 0.75 p. m. sodium.

The White Blood-corpuscles and the Blood-plates.

The White Blood-corpuscles, also called LEUCOCYTES or Lymphoid Cells, which occur in the blood in varying forms and sizes, form in a state of rest spherical lumps of a sticky, highly refractive power, capable of motion, non-membranous protoplasm, which show 1–4 nuclei on the addition of water or acetic acid. In human and mammalian blood they are larger than the red blood-corpuscles. They have also a lower specific gravity than the red

[1] Med. chem. Untersuch., S. 390 and 393.

[2] Pflüger's Archiv, Bdd. 51 and 52.

[3] Zeitschr. f. Biologie, Bd. 12, S. 206, 207.

[4] Maly's Jahresber., Bd. 18, S. 88.

corpuscles, move in the circulating blood nearer to the walls of the vessel, and have also a slower motion.

The number of white blood-corpuscles varies not only in the different blood-vessels, but also under different physiological conditions. As an average we have only 1 white corpuscle for 350–500 red corpuscles. According to the investigations of ALEX. SCHMIDT[1] and his pupils, the leucocytes are destroyed in great part on the discharge of the blood before and during coagulation, so that discharged blood is much poorer in leucocytes than the circulating blood. The correctness of this statement has been denied by other investigators.

From a histological standpoint we generally discriminate between the different kinds of colorless blood-corpuscles; chemically considered, however, there is no known essential difference between them. With regard to their importance in the coagulation of fibrin ALEX. SCHMIDT and his pupils distinguish between the leucocytes which are destroyed by the coagulation and those which are not. The last mentioned give with alkalies or common-salt solutions a slimy mass; the first do not show such behavior.

The protoplasm of the leucocytes has during life amœboid movements which partly make possible the wandering of the cells and partly the taking up of smaller grains or foreign bodies within the same. On these grounds the occurrence of *myosin* in them has been admitted even without any special proof thereof. ALEX. SCHMIDT[2] claims to have found *serglobulin* in equine-blood leucocytes which had been washed with ice-cold water. There are also certain leucocytes as above stated which yield a slimy mass when treated with alkalies or NaCl solutions, which seem to be identical with the so-called *hyaline substance* of ROVIDA found in the pus-cells. On digesting the leucocytes with water a solution of a proteid body is obtained which can be precipitated by acetic acid and which is not soluble in an excess of the acid and forms the chief mass of the leucocytes. This substance, which is undoubtedly related to coagulation, has been described under different names (see Chapter V), and consists, chiefly at least, of nucleohiston.

Glycogen has been found in the leucocytes by HOPPE-SEYLER,[3]

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[1] Pflüger's Archiv, Bd. 11.

[2] L.c.

[3] Physiol. Chem. Berlin, 1878–1881. S. 82.

Salomon,[1] Gabritschewsky,[2] and other investigators. The glycogen found by Huppert,[3] Czerny,[4] and others in the blood probably originated from the leucocytes. The constituents of the leucocytes are the same as the constituents of the cell as described in Chapter V.

The **blood-plates** (Bizzozero's), hæmatoblasts (Hayem), whose nature and physiological importance have been much questioned, are pale, colorless, gummy disks, round or more oval in shape and generally with a diameter two or three times smaller than the red blood-corpuscles. Certain investigators claim that the blood-plates occur preformed in the circulating blood, while others on the contrary deny this. According to Löwit[5] the blood-plates are formed from the leucocytes with the elimination of globulin substance, hence they are also called *globulin-plates*. According to Mosen these globulin-plates are not identical with the true blood-plates, and these first are derived very likely from the latter. The blood-plates separate into two substances by the action of different reagents, namely, one which is homogeneous and non-refractive, while the other is highly refractive and granular. Blood-plates readily stick together and attach themselves to foreign bodies.

According to the important researches of Kossel and Lilienfeld[6] the blood-plates consist of a chemical combination between proteid and nuclein, and hence they are called *nuclein-plates* by Lilienfeld. According to this investigator they are derivatives of the cell nucleus, a view which is in accord with Hlava's statements. It seems certain that the blood-plates stand in a certain relationship to the coagulation of blood, and according to Lilienfeld the fibrin coagulation is indeed a function of the cell nucleus. The importance of these formations to blood coagulation will be referred to later.

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[1] Deutsch. med. Wochenschr., 1877, Nos. 8 and 35.

[2] Arch. f. exp. Path. und Pharm., Bd. 28.

[3] Centralbl. f. Physiol., 1892, Part 14.

[4] Arch. f. exp. Path. und Pharm., Bd. 31.

[5] In regard to the literature of the blood-plates, see Lilienfeld, Du Bois-Reymond's Archiv, 1892, and R. Mosen, *ibid.*, 1893.

[6] L.c.; also Lilienfeld, "Leukocyten und Blutgerinnung," Verhandl. d. physiol. Gesellsch. zu Berlin, 1892.

III. The Blood as a Mixture of Plasma and Blood-corpuscles.

The blood in itself is a thick, sticky, lighter or darker red opaque liquid having a salty taste and a faint odor differing in different kinds of animals. On the addition of sulphuric acid to the blood the odor is more pronounced. In adult human beings the specific gravity ranges between 1.045 and 1.075. It has an average of 1.058 for grown men and a little less for women. According to SCHERRENZISS[1] the fœtal blood has a lower specific gravity than the blood of grown persons. LLOYD JONES[2] found that the specific gravity is highest at birth and lowest in children when about two years old and in pregnant women. The determinations of LLOYD JONES, HAMMERSCHLAG,[3] and others show that the variation of the specific gravity, dependent upon age and sex, corresponds to the variation in the quantity of hæmoglobin.

The determination of the specific gravity is most accurately done by means of the pyknometer. For clinical purposes where only small amounts are available it is best to proceed with the method as suggested by HAMMERSCHLAG. Prepare a mixture of chloroform and benzol of about 1.050 sp. gr. and add a drop of the blood to this mixture. If the drop rises to the surface then add benzol, and if it sinks add chloroform. Continue this until the drop of blood suspends itself midway and then determine the specific gravity of the mixture by means of an areometer.

The reaction of the blood is alkaline. The amount of alkali, calculated as Na_2CO_3, is in the dog about 2 (ZUNTZ[4]), in rabbits about 2.5 (LASSAR[5]), and in man 3.38–3.90 p. m. (v. JAKSCH[6]). The alkaline reaction diminishes outside of the body, and indeed the more quickly the greater the original alkalinity of the blood. This depends on the formation of acid in the blood, in which the red blood-corpuscles seem to take part in some way or another. After excessive muscular activity the alkalinity is diminished on account of the formation of acid in the muscles (PEIPER,[1] COHNSTEIN[2]), and it is also decreased after the continuous use of acids (LASSAR, FREUDBERG[3]).

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[1] Cit. from Maly's Jahresber., Bd. 18, S. 85.

[2] Journ. of Physiol., Vol. 8.

[3] Wien. klin. Wochenschr., 1890, and Zeitschr. f. klin. Med., Bd. 20.

[4] Centralbl. f. d. med. Wissensch., Bd. 5, S. 531 and 801.

[5] Pflüger's Archiv, Bd. 9.

[6] Zeitschr. f. klin. Med., Bd. 13, S. 350.

The color of the blood is red—light scarlet-red in the arteries and dark bluish-red in the veins. Blood free from oxygen is dichroitic, dark red by reflected light, and green by transmitted light. The blood-coloring matters occur in the blood-corpuscles. For this reason blood is opaque in thin layers and acts as a "deckfarbe." If the hæmoglobin is removed from the stroma and dissolved by the blood-liquid, by any of the above-mentioned means the blood becomes transparent and acts then like a "lake color." Less light is now reflected from its interior, and this laky blood is therefore darker in thicker layers. On the addition of salt solutions to the blood-corpuscles they shrink and more light is reflected and the color appears lighter. A great abundance of red corpuscles makes the blood darker, while by diluting with serum or by a greater abundance of white corpuscles the blood becomes lighter in appearance. The different colors of arterial and of venous blood depend on the varying quantity of gas contained in these two varieties of blood or, better, on the different amounts of oxyhæmoglobin and hæmoglobin they contain.

The most striking property of blood consists in its coagulating within a shorter or longer time, but as a rule very shortly after leaving the vein. Different kinds of blood coagulate with varying rapidity; in human blood the first marked sign of coagulation is seen in 2–3 minutes, and within 7–8 minutes the blood is thoroughly converted into a gelatinous mass. If the blood is allowed to coagulate slowly, the red corpuscles have time to settle more or less before the coagulation, and the blood-clot then shows an upper, yellowish-gray or reddish-gray layer consisting of fibrin enclosing chiefly colorless corpuscles. This layer has been called *crusta inflammatoria* or *phlogistica*, because it has been especially observed in inflammatory processes, and is considered one of the characteristics of them. This crusta or "*buffy coat*" is not characteristic of any special disease, and it occurs chiefly when the blood coagulates slowly or when the blood-corpuscles settle more quickly than usual. A buffy coat is often observed in the slow-coagulating equine blood. The blood from the capillaries is not supposed to have the power of coagulating.

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[1] Virchow's Arch., Bd. 116.

[2] *Ibid.*, Bd. 130, which has also references to the works of Minkowski, Zuntz, and Geppert.

[3] Virchow's Arch., Bd. 125.

Coagulation is retarded by cooling, by diminishing the oxygen and increasing the amount of carbon dioxide, which is the reason that venous blood and to a much higher degree blood after asphyxiation coagulates more slowly than arterial blood. The coagulation may be retarded or prevented by the addition of acids, alkalies, or ammonia, even in small quantities; by concentrated solutions of neutral alkali salts and alkaline earths, alkali oxalates and fluorides; also by egg-albumin, solutions of sugar or gum, glycerin, or much water; also by receiving the blood in oil. Coagulation may be prevented by the injection of an albumose solution or by an infusion of the leech into the circulating blood, but this infusion of the leech acts in the same way on blood just expelled. According to DASTRE[1] the coagulation of the blood of a dog may be gradually prevented by a series of bleedings and re-injection of the defibrinated blood. The reason for this non-coagulation lies in the lack of fibrinogen. The coagulation may be facilitated by raising the temperature; by contact with foreign bodies, to which the blood adheres; by stirring or beating it; by admission of air; by diluting with very small amounts of water; by the addition of platinum-black or finely powdered carbon; by the addition of laky blood, which does not act by the presence of dissolved blood-coloring matters, but by the stromata of the blood-corpuscles (WOOLDRIDGE[2]), and also by the addition of the leucocytes from the lymphatic glands, or a watery saline extract of the lymphatic glands, testicles, or thymus. The active constituent of such a watery extract is the nucleoproteid called *tissue fibrinogen* or *nucleohiston*.

An important question to answer is why the blood remains fluid in the circulation while it quickly coagulates when it leaves the circulation.

When the blood leaves the vein it comes under new, abnormal conditions. It cools off, comes in contact with the air, its motion stops, and it is deprived of the influence of the living walls of the vessels. That the cooling is not the reason of the coagulation is proved by the fact that cooling is a good means of retarding coagulation. That the contact with air is not essential is shown by the fact that when blood is collected over mercury, so that it cannot absorb or expel any gas, it likewise coagulates. That the cessation of the motion does not cause the coagulation follows, since blood collected over mercury coagulates whether it is shaken or not, and further from the fact that motion, such as beating the blood, facilitates the coagulation.

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[1] Compt. rend d. soc. biol., Tome 45, and Arch. de physiol., Sér. 5, Tome 5.

[2] Die Gerinnung des Blutes (published by M. V. Frey, Leipzig, 1891).

The reason why blood coagulates on leaving the body is therefore to be sought for in the influence which the walls of the living and entire blood-vessels exert upon it. These views are derived from the observations of many investigators. From the observations of HEWSON,[1] LISTER,[2] and FREDERICQ[3] it is known that when a vein full of blood is ligatured at the two ends and removed from the body, the blood may remain fluid for a long time. BRÜCKE[4] allowed the heart removed from a tortoise to beat at 0° C., and found that the blood remained uncoagulated for some days. The blood from another heart quickly coagulated when collected over mercury. In a dead heart, as also in a dead blood-vessel, the blood soon coagulates, and also when the walls of the vessel are changed by pathological processes.

What then is the influence which the walls of the vessels exert on the liquidity of the circulating blood? FREUND[5] has found that the blood remains fluid when collected by means of a greased canula under oil or in a vessel smeared with vaseline. If the blood collected in a greased vessel be beaten with a glass rod previously oiled, it does not coagulate, but it quickly coagulates on beating it with an unoiled glass rod or when it is poured into a vessel not greased. The non-coagulability of blood collected under oil has been confirmed later by HAYCRAFT and CARLIER.[6] FREUND found on further investigating that the evaporation of the upper layers of blood or their contamination with small quantities of dust causes a coagulation even in a vessel treated with vaseline. According to FREUND, it is this adhesion between the blood or between its form-elements and a foreign substance—and the diseased walls of the vessel also act as such—that gives the impulse towards coagulation, while the lack of adhesion prevents the blood from coagulating. This adhesion of the form-elements of the blood to certain foreign substances seems to induce changes which apparently stand in a certain relationship to the coagulation of the blood.

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[1] Hewson's works, ed. by Gulliver, London, 1876.

[2] Proc. Roy. Soc., Vol. 12.

[3] Recherches sur la constitution du plasma sanguin. Gand, 1878.

[4] Virchow's Archiv, Bd. 12.

[5] Wien. med. Jahrb., 1886.

[6] Journal of Anat. and Physiol., Vol. 22.

The views in regard to these changes are very contradictory. According to ALEX. SCHMIDT[1] and the DORPAT SCHOOL, an abundant destruction of the leucocytes takes place in coagulation, and important constituents for the coagulation of the fibrin pass into the plasma. According to LÖWIT[2] and other experimenters the essential is not a destruction of the leucocytes, but an elimination of constituents from the cells into the plasma. This process is called *plasmoschisis* by LÖWIT.

According to BIZZOZORO[3] and others, the leucocytes are not the starting-point in the fibrin formation, but rather the blood-plates. This view is in good accord with the recent investigations of LILIENFELD and MOSEN.[4] According to LILIENFELD the blood-plates are considered as derived from the cell nucleus and according to this author the fibrin coagulation is a function of the cell nucleus. This view is contradicted by GRIESBACH[5] because, as he claims, the nucleus cannot take part in the coagulation, but that in the first place a part of the cell body is destroyed by plasmoschises, and this even while the nucleus remains still intact.

WOOLDRIDGE[6] takes a very peculiar position in regard to this question, namely, he considers the form-elements as only of secondary importance in coagulation. As found by him, a peptone-plasma, which has been freed from all form-constituents by means of centrifugal force, yields abundant fibrin when it is not separated from a substance which precipitates on cooling. This substance, which WOOLDRIDGE has called A-fibrinogen, seems to be identical with LÖWIT's globulin-plates, and it consists in all probability of a nucleoproteid, which is perhaps identical with prothrombin as isolated by PEKELHARING.[1] As this nucleoproteid originates, acording to the unanimous view of several investigators, from the form-elements of the blood, either the blood-plates or leucocytes, WOOLDRIDGE's experiments do not seem to contradict the generally accepted view that the form-elements of the blood are of the greatest importance in the coagulation of the same.

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[1] Pflüger's Archiv, Bd. 11. The works of Alex. Schmidt are found in Arch. f. Anat. und Physiol., 1861, 1862; Pflüger's Arch., Bdd. 6, 9, 11, 13. See especially Alex. Schmidt, Zur Blutlehre (Leipzig, 1892), which also gives the work of his pupils.

[2] Wien. Sitzungsber., Bdd. 89 and 90, and Prager med. Wochenschr., 1889. Referred to in Centralbl. f. d. med. Wissensch., Bd. 28, S.265.

[3] Virchow's Arch., Bd. 90; Centralbl. f. d. med. Wissensch., 1882, S. 17, 161, 353, 563; *ibid.*, 1883; Virchow's Festschrift, 1891.

[4] L.c.

[5] Pflüger's Archiv, Bd. 50, and Centralbl. f. d. med. Wissensch., 1892, S. 497.

[6] L.c.

The views are greatly divided in regard to those bodies which are eliminated from the form-elements of the blood before and during coagulation.

According to ALEX. SCHMIDT[2] the leucocytes, like all cells, contain two chief groups of constituents, one of which accelerates coagulation, while the other retards or hinders it. The first may be extracted from the cells by alcohol, while the other cannot be extracted. Blood-plasma contains only traces of thrombin, according to SCHMIDT, but does contain its antecedent, prothrombin. The bodies which accelerate coagulation are neither thrombin nor prothrombin, but they act in this wise in that they split off thrombin from the prothrombin. On this account they are called *zymoplastic substances* by ALEX. SCHMIDT. The nature of these bodies is unknown, and according to LILIENFELD[3] KH_2PO_4 is found amongst them, and SCHMIDT has given no notice of their behavior to the lime-salts, which have been found to have zymoplastic activity by other investigators. The constituents of the cells which hinder coagulation and which are insoluble in alcohol-ether are compound proteids and have been called *cytoglobin* and *preglobulin* by SCHMIDT. The retarding action of these bodies may be suppressed by the addition of zymoplastic substances, and the yield of fibrin on coagulation in this case is much greater than in the absence of the compound proteid-retarding coagulation. This last supplies the material from which the fibrin is produced. The process is, according to SCHMIDT, as follows: The preglobulin first splits, yielding serglobulin, then from this the fibrinogen is derived, and from this latter the fibrin is produced. The object of the thrombin is twofold. The thrombin first splits the fibrinogen from the paraglobulin and then converts the fibrinogen into fibrin. ALEX. SCHMIDT is now agreed with most investigators that fibrin is produced by an enzymotic transformation of the fibrinogen, and the influence of the serglobulin, as observed by him on the quantity of fibrin formed, he explains now by the assumption that the fibrinogen is produced by the splitting of the serglobulin.

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[1] Ueber das Fibrinferment. Verhandl. d. kon. Akad. van. Wetensch. te Amsterdam, Deel 1, No. 3, 1892.

[2] Zur Blutlehre.

[3] Weitere Beiträge zur Kenntniss der Blutgerinnung. Berlin, 1893.

According to SCHMIDT the retarding action of the cells is prominent during life, while the accelerating action is especially pronounced outside of the body or by coming in contact with foreign bodies. The parenchymous masses of the organs and tissues, through which the blood flows in the capillaries, are those cell masses which serve to keep the blood fluid during life (ALEX. SCHMIDT).

LILIENFELD[1] has given further proofs as to the occurrence in the form-elements of the blood of bodies which accelerate or retard the coagulation. According to this author the nature of these bodies is very markedly different from SCHMIDT'S idea. While, according to SCHMIDT, the coagulation accelerators are bodies soluble in alcohol, and the proteids exhausted with alcohol only act retardingly on coagulation, LILIENFELD states that the substance which acts acceleratingly and retardingly on coagulation consists of a nucleoproteid, namely, nucleohiston. Nucleohiston readily splits into leuconuclein and histon, the first of which acts as a coagulation excitant, while the other, introduced into the blood-vascular system, either intravascular or extravascular, robs the blood of its property of coagulating. Introduced into the circulatory system the nucleohiston splits into its two components. It therefore causes extensive coagulation on one side and makes the remainder of the blood uncoagulable on the other.

LILIENFELD[2] is of the view that fibrinogen does not exist dissolved in the plasma of the circulating blood. It passes into the plasma on the disintegration of the leucocytes and originates from the substance of cell nuclei of the leucocytes. Nucleohiston may be directly transformed into fibrin. LILIENFELD'S theory at the present time is that on leaving the veins the leucocytes of the blood are destroyed or the nuclein substance passes into the plasma. This nuclein substance splits the fibrinogen into thrombosin and a substance which gives the biuret reaction. The thrombosin combines with the soluble calcium salts, forming fibrin. The leuconuclein is therefore the real coagulation exciter (not the fibrin ferment); the histon split from the nucleohiston has, on the contrary, a retarding action on coagulation. As the blood-plates contain nuclein, they as well as the leucocytes take an active part in the fibrin coagulation.

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[1] See Lilienfeld: Ueber Leukocyten und Blutgerinnung. Verhandl. d. physiol. Gesellsch. zu Berlin, No. 11, 1892; Ueber den flüssigen Zustand des Blutes, etc., *ibid.*, No. 16, 1892; and Weitere Beiträge zur Kentnisse der Blutgerinnung, *ibid.*, July, 1893.

[2] Zeitschr. f. physiol. Chem., Bd. 20.

Brücke showed long ago that fibrin left an ash containing calcium phosphate. The fact that calcium salts may facilitate or even cause a coagulation in liquids poor in ferment has been known for several years through the researches of the author,[1] Green,[2] Ringer, and Sainsbury.[3] The necessity of the lime-salts for coagulation was first shown positively by the important investigations of Arthus and Pagès.[4] In regard to the manner in which the lime-salts act we have only lately been able to come to a result.

Freund[5] has given the following explanation for the action of lime-salts: The alkali phosphates pass from the form-elements into the plasma, which is richer in lime-salts and forms calcium phosphate. If the quantity of calcium phosphate in the plasma or other coagulable liquid is so great that it cannot be kept completely in solution, then, according to Freund, the separation of the excess is the cause of a part of the proteids becoming insoluble, that is, a cause for coagulation. Weighty objections can be made against this view, and it is also confuted by Latschenberger[6] and Strauch.[7]

According to Pekelharing[8] the process is as follows: The prothrombin is converted into thrombin by the action of the soluble lime-salts and fluids otherwise capable of coagulation, which contains only prothrombin, but no thrombin can therefore be brought to coagulation by the addition of soluble lime-salts. Thrombin, according to PEKELHARING, is a lime combination of prothrombin, and the process of coagulation consists in that the thrombin carries the lime to the fibrinogen, which is converted into the insoluble combination of fibrin and lime. The thrombin is hereby reconverted into prothrombin, which again takes up lime to be transformed into thrombin, which gives up its lime to a new portion of fibrinogen, converting it into fibrin; and so on. This explanation of the process is only a hypothesis, but the formation of thrombin from a mother-substance by the action of soluble lime-salts is, on the contrary, a positively proven fact.

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[1] Nova Acta reg. Soc. Scient. Upsala, Ser. III, Vol. 10, 1879.

[2] Journ. of Physiol., Vol. 8.

[3] *Ibid.*, Vols. 11 and 12.

[4] M. Arthus, Recherches sur la Coagulation du sang., Paris, 1890; Arthus et Pagès: Nouvelle Theorie, etc., Arch. de Physiol. (5), Tome 2, 1890.

[5] Wien. med. Jahrb., 1888, S. 259.

[6] *Ibid.*, 1888, S. 479, and Wien. med. Wochenschr., 1889.

[7] Dissertation. Dorpat, 1889. Ref. Maly's Jahresber., Bd. 19.

[8] Virchow's Festschrift, Bd. 1, 1891.

It is a question whether the prothrombin exists in the plasma of the circulating blood or whether it is a body eliminated from the form-elements before coagulation. ALEX. SCHMIDT claims that the circulating plasma contains prothrombin, but PEKELHARING disclaims this. Blood-plasma obtained by means of leech infusion does not coagulate on the addition of lime-salts, but does on the addition of a prothrombin solution. The form-elements, especially the blood-plates, are particularly well preserved by such plasma; and according to PEKELHARING it is probable that the circulating plasma does not contain any mentionable amounts of prothrombin, and that this body emerges from the form-elements, perhaps the blood-plates, before coagulation.

In opposition to the view of ALEX. SCHMIDT, who considers the fibrin coagulation as an enzymotic process, WOOLDRIDGE[1] is of the opinion that the fibrin ferment is not the cause of the coagulation, but is a product of the chemical processes taking place during coagulation. WOOLDRIDGE claims, on the contrary, that lecithin and protein substances containing lecithin are of the greatest importance in the coagulation. This product is obtained by cooling the peptone-plasma which has been centrifugated, and the substance which separates has been called by WOOLDRIDGE *A*-fibrinogen. The plasma, according to WOOLDRIDGE, contains in itself all qualities necessary to produce a coagulation, and the form-elements are only of a secondary importance. Peptone-plasma which has been centrifugated and which is entirely free from form-elements, but contains the *A*-fibrinogen, coagulates on diluting with water, by the passage of carbon dioxide through the liquid, or after the addition of a little acetic acid, and the fibrin ferment is thereby formed. WOOLDRIDGE designates as *C*-fibrinogen the ordinary fibrinogen isolated by the method suggested on page 113. This fibrinogen occurs indeed in transudations, but it only occurs in the peptone-plasma in very small quantities. A third fibrinogen occurs in the greatest amounts in the peptone-plasma, and this is the mother-substance of the *C*-fibrinogen, and called *B*-fibrinogen by WOOLDRIDGE. The *B*-fibrinogen is converted into fibrin by lecithin and leucocytes from the lymphatic glands, but not by fibrin ferment or blood-serum. After the previous action of serum or fibrin ferment the *B*-fibrinogen yields fibrin on diluting with water. The one most essential for the fibrin coagulation is, according to WOOLDRIDGE, a reciprocal action between *A*- and *B*-fibrinogen. An exchange of lecithin from the *A*-fibrinogen to the *B*-fibrinogen takes place.

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[1] The summary of the observations of Wooldridge are found in the pre-[illegible] publ[illegible] "Die Gerinnung des Blutes" (M. v. Frey, 1891).

HALLIBURTON[1] has opposed weighty arguments to this theory. It is also difficult to find in WOOLDRIDGE's discussion conclusive proofs for the above views, and the experiments by which they are supported are interpreted with difficulty. On account of the very complicated condition of the question of coagulation at the present time, it is impossible to draw any definite conclusions from the observations of WOOLDRIDGE.

Intravascular coagulation. It has been shown by ALEX. SCHMIDT and his students, as also by WOOLDRIDGE, WRIGHT,[2] and others, that an intravascular coagulation may be brought about by the intravenous injection into the circulating blood of a large quantity of a thrombin solution, as also by the injection of leucocytes or tissue fibrinogen (impure nucleohiston). In rabbits this coagulation may extend through the entire vascular system, while in dogs it is ordinarily confined to the portal system. The blood in the other parts of the vascular system has generally a decreased coagulat ability. If too little of the above-mentioned bodies be injected, then we only observe a marked retarding tendency in the coagulation of the blood. According to WOOLDRIDGE we can generally maintain that after a short stage of accelerated coagulability, which may lead to a total or partial intravascular coagulation, a second stage of a diminished or even arrested coagulability of the blood follows. The first stage is designated as the *positive* and the other the *negative phase* of coagulation. These statements have been confirmed by several investigators.

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[1] Journal of Physiol., Vol. 9.

[2] A study of the intravascular coagulation, etc., Proceed. of the Roy. Irish Acad. (3), Vol. 2; see also Wright: Lecture on tissue or cellfibrinogen, The Lancet; 1892; and Wooldridge's Method of producing immunity, etc., Brit. Med. Journal, Sept. 1891.

There is no doubt that the positive phase is brought about by an abundant introduction of thrombin, or by a rapid and abundant formation of the same. According to ALEX. SCHMIDT, the zymoplastic substances soluble in alcohol are active in these processes, while according to the investigations of PEKELHARING this action is caused more likely by the leuconucleins, split off from the nucleohiston. According to WOOLDRIDGE, his tissue fibrinogen does not produce any intravascular coagulation if it is freed from contaminating bodies by means of alcohol. This corresponds with the statements of ALEX. SCHMIDT, but still further investigations are necessary.

In regard to the origin of the negative phase, attention has been called to histon, which has a retarding action on coagulation, and which is split off from the nucleohiston. According to WRIGHT and PEKELHARING, the retarding substances are albumoses, which are formed in the decomposition of the nucleoproteids. Albumoses have been detected by these investigators in the blood of animals during this phase, and also in the urine after intravenous injection of tissue fibrinogen. According to PEKELHARING, the albumoses act by combining with the calcium of the blood, and in this wise preventing coagulation. GROSJEAN[1] has found that blood which has regained its property of coagulation 24 hours after an albumose injection will not have its coagulation prevented by a fresh injection of albumose, hence it is immune against albumose injection. He also infers from these experiments that the albumose, to have a preventing action at all, must first undergo a change in the organism. This has been further studied by CONTEJEAN,[2] who finds that under the influence of injected albumose a special substance is secreted in the animal body which prevents coagulation. This seems to be brought about by means of the liver and intestine. A dog may be made immune against the preventive action of albumose by previously injecting a small quantity of "peptonized blood" into the vessels. The body hereby loses its property of producing substances which prevent coagulation under the influence of injected albumoses.

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[1] Travaux du laboratoire de L. Fréderiq. Tome 4. Liège, 1892.

[2] Arch. de Physiol., Sér. 5, Tome 7.

WRIGHT gives as reason why the intravascular coagulation of the blood of a dog is ordinarily confined to the portal system, in the fact that it contains larger quantities of carbon dioxide. An increased quantity of carbon dioxide in the blood favors the appearance of the positive phase, and an intravascular coagulation may be produced in dogs, who are asphyxiated by clamping the trachea, by injecting tissue fibrinogen (impure nucleohiston).

The *gases of the blood* will be treated of in Chapter XVII (on respiration).

IV. The Quantitative Composition of the Blood.

The quantitative analysis of blood cannot be of value for the blood as an entirety. We must ascertain on one side the relationship of the plasma and blood-corpuscles to each other, and on the other side the constitution of each of these two chief constituents. The difficulties which stand in the way of such a task, especially in regard to the living, non-coagulated blood, have not been removed. Since the constitution of the blood may differ not only in different vascular regions, but also in the same region under different circumstances, which renders also a number of blood analyses necessary, it can hardly appear remarkable that our knowledge of the constitution of the blood is still relatively limited.

The relative volume of blood-corpuscles and serum in defibrinated blood may be determined, according to L. and M. BLEIBTREU,[1] by various methods if the defibrinated blood is mixed with different proportions of NaCl solutions of 6 p. m. (1 vol. salt solution to 1 vol. blood), the blood-corpuscles allowed to settle to the bottom or facilitated by centrifugal force, and the clear supernatant mixture of serum and common-salt solution siphoned off. The methods are as follows:

1. Determine the quantity of nitrogen in at least two different portions of the mixture of serum and salt solution by means of KJELDAHL's method and calculate the quantity of proteid corresponding thereto by multiplying with 6.25, and the relative volume of blood x and also the volume of the structural elements $(1 - x)$ is found by the following equation:

$$(e_1 - e_2)x = \frac{s_2}{b_2}e_2 - \frac{s_1}{b_1}e_1.$$

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[1] Pflüger's Archiv, Bd. 51.

In this equation (for mixtures 1 and 2), b_1 or b_2 represents the volume of blood in the mixture, s_1 or s_2 the volume of salt solution, and e_1 or e_2 the quantity of proteid in a certain volume of each mixture.

2. Determine the specific gravity of the blood-serum, the salt solution and at least one of the mixtures of serum and salt solution by means of a pyknometer. The relative volume of serum x is found in this by the following equations:

$$x = \frac{s}{b} \cdot \frac{S - K}{S_2 - K}.$$

In this equation s and b represent the volumes of salt solution and blood mixed. S represents the specific gravity of the obtained serum and salt solution obtained on allowing the blood-corpuscles to settle, S_2 the sp. gr. of the serum, and K that of the salt solution.

For horses' blood, two other, shorter methods may be made use of (see the original article).

HAMBURGER[1] raises special objections to the above methods, but according to BLEIBTREU they are of no practical importance as long as the blood is not diluted with more than an equal volume of the salt solution.[2]

EYKMAN[3] and HEDIN[4] have raised important objections to BLEIBTREU'S method. They have shown by different methods that the red corpuscles are not changed in volume only in such salt solutions which are isotonic with the plasma or serum. (In regard to the osmotic pressure of the blood-corpuscles and the isotonic relationship of salt solutions and serum, see HAMBURGER[5].) Such a solution is not one containing 6 p. m. NaCl, for human, ox, or horse's blood, but rather one containing about 9 p. m. NaCl (LACKSCHEWITZ[6]). The blood-corpuscles swell up in a solution of 6 p. m. NaCl, and therefore an abundant exchange of constituents takes place between them and the salt solution; hence BLEIBTREU'S method is incorrect. HEDIN, as before him BIERNACKI,[7] could not obtain corresponding results of the volume of corpuscles calculated from the nitrogen determined. The question arises whether this method is available if we use an isotonic salt solution. According to HEDIN this is not true, as he has found that the red blood-corpuscles take up considerable quantities of plasma proteid, even in isotonic common-salt solution, without changing their volume. This statement is disputed by M. BLEIBTREU,[1] and the analyses made by using an isotonic salt solution, although not numerous, have led to very good results.

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[1] Centralbl. f. Physiol., Bd. 7, S. 161.

[2] See M. Bleibtreu, Pflüger's Archiv, Bd. 55.

[3] Pflüger's Arch., Bd. 60.

[4] *Ibid.*, and Skand. Arch. f. Physiol., Bd. 5.

[5] Virchow's Arch., Bd. 140, S. 505.

[6] Pflüger's Arch., Bd. 59.

[7] Zeitschr. f. physiol. Chem., Bd. 19.

For clinical purposes the relative volume of corpuscles in the blood may be determined by the use of a small centrifuge called *hæmatocrit*, constructed by BLIX and described and tested by HEDIN.[2] A measured quantity of blood is mixed with a known volume (best an equal volume) of a fluid which prevents coagulation. This mixture is introduced into a tube and then centrifuged. HEDIN uses MÜLLER'S solution as a diluting fluid and DALAND[3] a 2.5% solution of potassium bichromate. After complete centrifugation the layer of blood-corpuscles is read off on the graduated tube, and the volume of blood-corpuscles calculated in 100 vols. of the blood therefrom. By means of comparative counts HEDIN and DALAND have found that an approximately constant relation exists between the volume of the layer of blood-corpuscles and the number of red corpuscles under physiological conditions, so that the number of corpuscles may be calculated from the volume. DALAND has shown that such a calculation gives approximate results also in disease, when the size of the blood-corpuscles does not essentially deviate from the normal. In certain diseases, such as pernicious anæmia, this method gives such inaccurate results that it cannot be used. The uselessness of this method for the exact estimation of the volume of blood-corpuscles has been demonstrated[4] by L. BLEIBTREU.[5] EYKMAN as well as HEDIN repudiate the objections made by BLEIBTREU against the hæmatocrit method; but they also show that MÜLLER'S solution as well as the 2.5% potassium bichromate solution causes the blood-corpuscles to swell up, and hence lead to incorrect results. According to HEDIN, in working with the hæmatocrit dilute the blood, which is kept fluid by a 1 p. m. oxalate solution, with an equal volume of a solution containing 9 p. m. NaCl. Under these conditions the determination of the volume of blood-corpuscles by the hæmatocrit method is very serviceable. This method is not available for the exact determination of the volume of corpuscles, because the sediment of blood-corpuscles to all appearances does not consist only of blood-corpuscles, but also some plasma.

If we know the relationship between the volume of corpuscles and blood liquid we can also estimate the relative weights by determining the specific gravity of the blood and serum. In direct determinations of the proportion by weight we proceed in the following way:

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[1] Pflüger's Arch., Bd. 60.

[2] Skandinav. Arch. f. Physiol., Bd. 2, S. 134 and 361.

[3] Fortschritte d. Med., Bd. 9, 1891.

[4] Biernacki, Zeitschr. f. physiol. Chem., Bd. 19.

[5] Berl. klin. Wochenschr., 1893, No. 30.

If any substance is found in the blood which belongs exclusively to the plasma and does not occur in the blood-corpuscles, then the amount of plasma contained in the blood may be calculated if we determine the amount of this substance in 100 parts of the plasma or serum, respectively, on one side and in 100 parts of the blood on the other. If we represent the amount of this substance in the plasma by p and in the blood by b, then the amount of x in the plasma from 100 parts of blood is $x = \frac{100 . b}{p}$.

Such a substance, which occurs only in the plasma, is fibrin according to HOPPE-SEYLER,[1] sodium according to BUNGE[2] (in certain kinds of blood), and sugar according to OTTO.[3] The experimenters just named have tried to determine the amount of the plasma and blood-corpuscles, respectively, in different kinds of blood, starting from the above-mentioned substances.

Another method, suggested by HOPPE-SEYLER,[4] is to determine the total amount of hæmoglobin and proteids in a portion of blood, and on the other hand the amount of hæmoglobin and proteids in the blood-corpuscles (from an equal portion of the same blood), which have been sufficiently washed with common-salt solution by centrifugal force. The figures obtained as a difference between these two determinations correspond to the amount of proteids which was contained in the serum of the first portion of blood. If we now determine the proteids in a special portion of serum of the same blood, then the amount of serum in the blood is easily determined. The usefulness of this method has been confirmed by BUNGE by the control experiments with the sodium determinations. If the amount of serum and blood-corpuscles in the blood is known, and we then determine the amount of the different blood-constituents in the blood-serum on one side and of the total blood on the other, the distribution of these different blood-constituents in the two chief components of the blood, plasma, and blood-corpuscles may be ascertained. According to the just-mentioned procedure, the following analyses of pig's blood and ox's blood have been made by BUNGE. The analyses of human blood have been made by C. SCHMIDT[5] according to another method, which perhaps have given rather too high results for the weight of the blood-corpuscles. All figures represent parts in 1000 parts of blood.

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[1] Handb. d. physiol. und pathol. chem. Analyse, 6. Aufl., S. 417.

[2] Zeitschr. f. Biologie, Bd. 12.

[3] Pflüger's Archiv, Bd. 35, S. 480–482.

[4] See Handb. d. physiol. und pathol. chem. Analyse, 6. Aufl.

[5] Cited and partly recalculated from v. Gorup-Besanez, Lehrb. der physiol. Chem., 4. Aufl., S. 345.

	Pig's Blood.		Ox's Blood.		Human Blood.			
					Man's		Woman's.	
	Blood-corpuscles 436.8	Serum 563.2	Blood-corpuscles 318.7	Serum 681.3	Blood-corpuscles 513.02	Serum 486.98	Blood-corpuscles 396.24	Serum 603.76
Water	276.100	517.900	191.200	622.200	349.690	439.020	272.560	551.990
Solids	160.700	45.300	127.500	59.100	163.330	47.960	123.680	51.770
Hæmoglobin and Proteid	151.600	38.100	123.600	49.900	159.590	43.820	120.130	46.700
Remaining org. bodies.	5.200	2.800	2.400	3.800				
Inorganic bodies	3.900	4.300	1.500	5.400	3.740	4.140	3.550	5.070
K_2O	2.421	0.154	0.238	0.173	1.586	0.153	1.412	0.200
Na_2O	....	2.406	0.667	2.964	0.241	1.661	0.648	1.916
CaO	....	0.072	....	0.070	....	....	....	....
MgO	0.069	0.021	0.005	0.031	....	....	....	....
Fe_2O_3	....	0.006	....	0.007	....	....	....	....
Cl	0.657	2.034	0.521	2.532	0.898	1.722	0.362	0.144
P_2O_5	0.903	0.106	0.224	0.181	0.695	0.071	0.643	2.202

HOPPE-SEYLER, SACHARJIN,[1] and OTTO[2] found 584.9–693.5 p. m. plasma and 415.1–306.5 p. m. blood-corpuscles in horse's blood. BUNGE[3] found, on the contrary, in an analysis 468.5 p. m. serum and 531.5 p. m. blood-corpuscles—more blood-corpuscles, therefore, than serum. For human blood ARRONET[4] has found 478.8 p. m. blood-corpuscles and 521.2 p. m. serum (in defibrinated blood) as an average of nine determinations. SCHNEIDER[5] found 349.6 and 650.4 p. m. respectively in women.

The relationship between blood-corpuscles and plasma varies; in the blood of men it is about 50% of the weight of the blood, while in women it is somewhat more. The quantity of plasma in animals is often greater, and in certain cases it may indeed be two thirds of the weight of the blood. The relationship between the corpuscular elements and the plasma may undergo marked fluctuation. L. and M. BLEIBTREU found in 10 experiments with defibrinated horse-blood that the relative volume of form-elements varied between 261.4 and 409.5 p. m. The relative volume of blood liquid to the corpuscular elements varies according to the manner in which the blood is drawn from the animal. L. and M. BLEIBTREU[6] have found that the blood from a killed animal is regularly richer in corpuscles than blood taken from the veins. Water occurs in the greatest amount in the plasma or serum, which latter ordinarily contains at least $\frac{9}{10}$ water, while the blood-corpuscles contains only a little more than $\frac{1}{2}$ or about $\frac{2}{3}$ water. Iron probably occurs only in the blood-corpuscles. Chlorine and sodium prevail in the plasma, while potassium and phosphoric acid prevail in the blood-corpuscles. In a few varieties of blood (pig's and horse's blood) the sodium is found exclusively in the plasma or serum, the potassium prevailing in the blood-corpuscles (Bunge[1]). In dog's and ox's blood the blood-corpuscles are, however, richer in sodium than in potassium (Bunge). In man the potassium exists in large quantities in the blood-corpuscles and only in very small quantities in the plasma (C. Schmidt,[2] Wanach[3]). The alkaline earths occur chiefly in the plasma. Manganese has also been found in the blood, as well as traces of lithium, copper, lead, and silver. The blood as a whole contains in ordinary cases 770–820 p. m. water, with 180–230 p. m. solids; of these 173–220 p. m. are organic and 6–10 p. m. inorganic. The organic consist, deducting 6–12 p. m. extractive bodies, of proteids and hæmoglobin. The amount of this last-mentioned body in human blood is about 130–150 p. m. The quantity of hæmoglobin in dog's blood is about the same; and Bunge found 114 p. m. hæmoglobin in pig-blood and 89.4 p. m. in ox-blood.

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[1] Hoppe-Seyler's Physiol. Chem., 1877–1881, S. 447.

[2] Pflüger's Archiv, Bd. 35.

[3] l. c.

[4] Maly's Jahresber., Bd. 17, S. 139.

[5] Centralbl. f. Physiol., Bd. 5, S. 362.

[6] L. c.

The amount of sugar in the blood is on an average 1–1.5 p. m. The quantity of urea, which varies between 0.2 and 1.5 p. m., is greater after partaking of food than during fasting (Gréhant and Quinquaud,[4] Schöndorff[5]). The quantity of uric acid may be 0.1 p. m. in bird's blood (v. Schroeder[6]). Lactic acid was first found in human blood by Salomon and then by Gaglio, Berlinerblau and Irisawa.[7] The quantity of lactic acid may vary considerably. Berlinerblau found 0.71 p. m. as maximum.

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[1] L. c.

[2] L. c.

[3] Maly's Jahresber., Bd. 18, S. 88.

[4] Journal de l'anatomie et de la physiol., Tome 20, and Compt. rend., Tome 98.

[5] Pflüger's Arch., Bd. 54.

[6] Ludwig's Festschrift, 1887, p. 89.

[7] Zeitschr. f. physiol. Chem., Bd. 17, which also gives the older literature.

The Composition of the Blood in Different Vascular Regions and under Different Physiological Conditions.

Arterial and Venous Blood. The most striking difference between these two kinds of blood is the variation in color caused by their containing different amounts of gas and different amounts of oxyhæmoglobin and hæmoglobin. The arterial blood is light red; the venous blood is dark red, dichroitic, greenish by transmitted light through thin layers. The arterial coagulates more quickly than the venous blood. The latter, on account of the transudation which takes place in the capillaries, is somewhat poorer in water but richer in blood-corpuscles and hæmoglobin than the arterial blood, but this is denied by modern investigators. According to KRÜGER[1] and his pupils the quantity of dry residue and hæmoglobin in blood from the carotid artery and from the jugular vein (in cats) are the same. RÖHMANN and MÜHSAM[2] could not detect any difference in the quantity of fat in arterial and venous blood.

Blood from the Portal Vein and the Hepatic Vein. The blood of the hepatic vein is poorer in ordinary red blood-corpuscles but richer in white and so-called young red blood-corpuscles. A few investigators have concluded from this that a formation of red blood-corpuscles takes place in the liver, while others claim that a destruction takes place.

In consequence of the small quantities of bile and lymph found relatively to the large quantity of blood circulating through the liver in a given time, we can hardly expect to detect a positive difference in the composition between the blood of the portal and hepatic veins by chemical analysis. The statements in regard to such a difference are in fact contradictory. For example, DROSDOFF[3] has found more hæmoglobin in the hepatic than in the portal vein, while OTTO[4] found less. KRÜGER[5] finds that the quantity of hæmoglobin, as well as the solids, in the blood from the vessels passing to and from the liver is different, but a constant relationship cannot be determined. The disputed question as to the varying quantities of sugar in the portal and hepatic veins will be discussed in a following chapter (see Chapter VIII, on the formation of sugar in the liver). After a meal rich in carbohydrates the blood of the portal vein not only becomes richer in dextrose, but may contain also dextrin and other carbohydrates (v. MERING,[1] OTTO[2]). The amount of urea in the blood from the hepatic vein is greater than in other blood (GRÉHANT and QUINQUAUD[3]).

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[1] Zeitschr. f. Biologie, Bd. 26.

[2] Pflüger's Archiv, Bd. 46.

[3] Zeitschr. f. physiol. Chem., Bd. 1.

[4] Christiania Videnskabs. Selskabs Forhandlinger, 1886, No. 11. See Maly's Jahresber., Bd. 17, S. 134.

[5] Zeitschr. f. Biologie, Bd. 26.

Blood of the Splenic Vein is decidedly richer in leucocytes than the blood from the splenic artery. The red blood-corpuscles of the blood from the splenic vein are smaller than the ordinary, less flattened, and show a greater resistance to water. The blood from the splenic vein is also claimed to be richer in water, fibrin, and albumin than the ordinary venous blood (BÉCLARD[4]). According to v. MIDDENDORFF,[5] it is richer in hæmoglobin than arterial blood. KRÜGER[6] and his pupils have found that the blood from the vena lienalis is generally richer in hæmoglobin and solids than arterial blood; still the contrary is often found. The blood from the splenic vein coagulates slowly.

The Blood from the Veins of the Glands. The blood circulates with greater rapidity through a gland during activity (secretion) than when at rest, and the outflowing venous blood has therefore during activity a lighter red color and a greater amount of oxygen. Because of the secretion the venous blood also becomes somewhat poorer in water and richer in solids.

The blood from the *Muscular Veins* shows an opposite behavior, for during activity it is darker and more venous in its properties because of the increased absorption of oxygen by the muscles and still greater production of carbon dioxide than when at rest.

Menstrual Blood has, according to an old statement, not the power of coagulating. This statement is nevertheless false, and the apparent uncoagulability depends in part on the womb and the vagina retaining the blood-clot, so that only fluid cruor is at times eliminated, and in part on a contamination with vaginal mucus which disturbs the coagulation.

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[1] Du Bois-Reymond's Archiv, 1887, S. 412 and 431.

[2] See note 4, page 169.

[3] Journal d. l'anatomie et de la physiol., Tome 20, and Compt. rend., Tome 98.

[4] Arch. générale de médecine, Tome 18.

[5] Cit. from Centralbl. f. Physiol., Bd. 2, S. 753.

[6] Zeitschr. f. Biologie, Bd. 26.

The Blood of the Two Sexes. Woman's blood coagulates somewhat more quickly, has a lower specific gravity, a greater amount of water, and a smaller quantity of solids than the blood of man. The amount of blood-corpuscles and hæmoglobin is somewhat smaller in woman's blood. The amount of hæmoglobin is, according to OTTO, 146 p. m. for man's blood and 133 p. m. for woman's.

During *pregnancy* NASSE[1] has observed a decrease in the specific gravity, with an increase in the amount of water until the end of the eighth month. From then the specific gravity increases, and at delivery it is normal again. The amount of fibrin is somewhat increased (BECQUEREL and RODIER,[2] NASSE). The number of blood-corpuscles seems to decrease. In regard to the amount of hæmoglobin the statements are somewhat contradictory. COHNSTEIN[3] found the number of red corpuscles diminished in the blood of pregnant sheep as compared to non-pregnant, but the red corpuscles were larger, and the quantity of hæmoglobin in the blood was greater in the first case.

The Blood at Different Periods of Life. Fœtal blood is strikingly poorer in blood-corpuscles and hæmoglobin than the blood of the adult. The fœtal blood at the moment of birth has, according to SCHERRENZISS,[4] a lower specific gravity, a markedly lower amount of hæmoglobin, and a little less fibrin, but a greater amount of mineral bodies, especially proportionally more sodium (but less potassium) than the blood of adults. A few hours after birth the blood of the child has the same or greater quantity of hæmoglobin than the blood of the mother (COHNSTEIN, ZUNTZ,[5] OTTO[6]). The quantity of hæmoglobin and blood-corpuscles quickly increases after birth; still they do not both increase at the same rate, as the amount of hæmoglobin increases much faster. Two or three days after birth the hæmoglobin reaches a maximum (20–21%), which is greater than at any other period of life. This is the cause of the great abundance of solids in the blood of new-born infants as observed by several investigators. The quantity of hæmoglobin and blood-corpuscles sinks gradually from this first maximum to a minimum of about 11% hæmoglobin, which minimum appears in human beings between the fourth and eighth years. The quantity of hæmoglobin then increases again until about the twentieth year, when a second maximum of 13.7–15% is reached. The hæmoglobin remains at this point only towards the forty-fifth year, and then gradually and slowly decreases (LEICHTENSTERN,[1] OTTO[2]). According to older statements, the blood at old age is poorer in blood-corpuscles and albuminous bodies but richer in water and salts.

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[1] Maly's Jahresber., Bd. 7, S. 129.

[2] Traité de chimie pathol. Paris, 1854. P. 59.

[3] Pflüger's Archiv, Bd. 34, S. 233.

[4] Maly's Jahresber., Bd. 18.

[5] Pflüger's Arch., Bd. 34, S. 173.

[6] Maly's Jahresber., Bdd. 15 and 17.

The Influence of Food on the Blood. In complete starvation no decrease in the amount of solid blood constituents is found to take place (PANUM[3] and others). The amount of hæmoglobin is a little increased (SUBBOTIN,[4] OTTO), and also the number of red blood-corpuscles increases (WORM MÜLLER,[5] BUNTZEN[6]), which probably depends on the fact that the blood-corpuscles are not so quickly transformed as the serum. As after-effect the inanition causes an anæmic condition.

After a rich meal the relative number of blood-corpuscles, especially after secretion of digestive juices or absorption of nutritive liquids, may be increased or diminished (BUNTZEN, LEICHTENSTERN). The number of colorless blood-corpuscles may be increased to such an extent, after a diet rich in proteids, that a true digestion leucocytosis appears (HOFMEISTER and POHL[7]). After a diet rich in fat the plasma becomes, even after a short time, more or less milky-white, like an emulsion. The constitution of the food acts essentially on the amount of hæmoglobin in the blood. The blood of herbivora is generally poorer in hæmoglobin than that from carnivora, and SUBBOTIN has observed in dogs after a partial feeding with food rich in carbohydrates that the amount of hæmoglobin sank from the physiological average of 137.5 p. m. to 103.2–93.7 p. m. According to LEICHTENSTERN a gradual increase in the amount of hæmoglobin is found to take place in the blood of human beings on enriching the food, and according to the same investigator the blood of lean persons is generally somewhat richer in hæmoglobin than blood from fat ones of the same age. The addition of iron salts to the food greatly influences the number of blood-corpuscles and especially the amount of hæmoglobin they contain. The action of the iron salts is obscure. According to BUNGE[1] they probably combine with the sulphuretted hydrogen of the intestinal canal and thereby prevent the iron, associated in the food as protein combination, from being eliminated as iron sulphide.

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[1] Untersuch. über den Hämoglobingehalt des Blutes im gesunden und kranken Zustande. Leipzig, 1878.

[2] Maly's Jahresber., Bd. 17.

[3] Virchow's Arch., Bd. 29.

[4] Zeitschr. f. Biologie, Bd. 7.

[5] Transfusion und Plethora. Christiania, 1875.

[6] Om Ernæringens og Blodtabets Indflydelse på Blodet. Kjöbenhavn, 1879. See also Maly's Jahresber., Bd. 9.

[7] Arch. f. exp. Path. und Pharm., Bd. 25.

The Composition of the Blood under Abnormal Conditions may be changed either by the appearance of a foreign substance or by the quantities of any one or more of the blood constituents being abnormally increased or diminished. Changes of this last kind occur frequently.

An *increase in the number of red corpuscles*, a true "PLETHORA POLYCYTHÆMICA," takes place after transfusion of blood of the same species of animal. According to the observations of PANUM[2] and WORM MÜLLER,[3] the blood-liquid is quickly eliminated and transformed in this case,—the water being eliminated principally by the kidneys, and the albumin burned into urea, etc.,—while the blood-corpuscles are preserved longer and cause a "POLYCYTHÆMIA." A relative increase in the number of red corpuscles is found after abundant transudations from the blood, as in cholera and heart-failure, with considerable accumulation.

A *decrease in the number of red corpuscles* occurs in anæmia from different causes. Very excessive hemorrhage causes an acute anæmia or more correctly oligæmia. Even during the hemorrhage the remaining blood becomes richer in water by diminished secretion and excretion, as also by an abundant absorption of parenchymous fluid somewhat poorer in proteids and strikingly poorer in red blood-corpuscles. The oligæmia passes soon into a hydræmia. The amount of proteid then gradually increases again; but the re-formation of the red blood-corpuscles is slower, and after the hydræmia follows also an oligocythæmia. After a little time the number of blood-corpuscles rises to normal; but the re-formation of hæmoglobin does not keep pace with the re-formation of the corpuscles, and a chlorotic condition may appear. A considerable decrease in the number of red corpuscles occurs also in chronic anæmia and chlorosis; still in such cases an essential decrease in the amount of hæmoglobin occurs without an essential decrease in the number of blood-corpuscles. The decrease in the amount of hæmoglobin is more characteristic of chlorosis than a decrease in the number of red corpuscles.

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[1] Zeitschr. f. Physiol. Chem., Bd. 9.

[2] Virchow's Arch., Bd. 29.

[3] Transfusion und Plethora. Christiania, 1875.

A very considerable decrease in the number of red corpuscles (300,000–400,000 in 1 c.mm.) and diminution in the amount of hæmoglobin ($\frac{1}{5}$–$\frac{1}{10}$) occurs in pernicious anæmia (HAYEM, LAACHE[1]). On the contrary, the individual red corpuscles are larger and richer in hæmoglobin than they ordinarily are, and the number stands in an inverse relationship to the amount of hæmoglobin (HAYEM). Besides this the red corpuscles often, but not always, show in pernicious anæmia remarkable and extraordinary irregularities of form and size, which QUINCKE[2] has termed *poikilocytosis.*

The Composition of the Red Corpuscles. Irrespective of the changes in the amount of hæmoglobin, as just mentioned, the composition of the blood-corpuscles may be changed in other ways. By abundant transudations, as in cholera, the blood-corpuscles may give up water, potassium, and phosphoric acid to the concentrated plasma and become correspondingly richer in organic substances (C. SCHMIDT[3]). By a few other transudation processes, as in dysentery and dropsy with albuminuria, a considerable amount of proteid passes from the blood; the plasma becomes richer in water, and the blood-corpuscles take up water and so become poorer in organic substance (C. SCHMIDT).

The *number of leucocytes* may, as above mentioned, increase considerably under physiological conditions, such as after a meal rich in proteids (physiological leucocytosis). Under pathological conditions a *hyperleucocytosis* may occur, and according to VIRCHOW[4] this occurs in all pathological processes in which the lymphatic glands take part. Leucocytosis occurs prominently in leucæmia, which is characterized by the very great abundance of leucocytes in the blood. The number of leucocytes is not only absolutely increased in this disease, but also in proportion to the number of red blood-corpuscles, which is considerably diminished in leucæmia. The blood from a leucæmic patient has a lower specific gravity than the ordinary (1.035–1.040) and a lighter color, as if it were mixed with pus. The reaction is alkaline, but after death is often acid, probably due to a decomposition of the considerably increased lecithin. In leucæmic blood, volatile fatty acids, lactic acid, glycero-phosphoric acid, large amounts of xanthin bodies (SALOMON,[1] KOSSEL[2]), and the so-called CHARCOT's crystals (see Chapter XIII) have been found.

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[1] Die Anaemie. Christiania, 1883.

[2] Deutsch. Arch. f. klin. Med., Bdd. 20 and 25.

[3] Cit. from Hoppe-Seyler's Physiol. Chem., 1877–1881.

[4] Virchow's Gesammelte Abhandl. zur wissensch. Med., Bd. 8.

The *quantity of water* in the blood is increased in general dropsy, with or without kidney disease, in different forms of anæmia, in scurvy, and in febrile diseases. The amount of water is diminished in abundant transudations, by powerful laxatives, in diarrhœa, and especially in cholera.

The *amount of proteids* in the blood may be relatively increased (HYPERALBUMINOSIS) in cholera and after the action of laxatives. A decrease in the amount of proteids (HYPALBUMINOSIS) occurs after direct loss of proteids from the blood, as in hemorrhage, albuminuria, dysentery, copious formation of pus, etc., etc. The amount of *fibrin* is increased (HYPERINOSIS) in inflammatory diseases, pneumonia, acute muscular rheumatism, and erysipelas, in which the blood yields a "CRUSTA PHLOGISTICA" because it coagulates more slowly. The statements in regard to the occurrence of a hyperinosis in scurvy and hydræmia seems to require further confirmation. A decrease in the amount of fibrin (HYPINOSIS) has not been observed with certainty in any disease.

The *amount of fat in the blood* (LIPÆMIA) increases, irrespective of the increase after a diet rich in fat, in drunkards, in corpulent individuals, after fracture of the bones, and also in diabetes. In the last-mentioned case the increase in fat depends, according to HOPPE-SEYLER,[3] upon defective digestion. An increase in the amount of fat in the blood has also been observed in diseases of the liver, Bright's disease, tuberculosis, malaria, and cholera. v. JAKSCH[4] has observed volatile fatty acids in the blood (LIPACIDÆMIA) in febrile diseases and sometimes in diabetes.

The *amount of salts* in the blood is increased in dropsy, dysentery, and in cholera immediately after the first violent attack, but diminishes later after the attack in cholera, in scurvy, and in inflammatory diseases. The decrease of alkali salts, especially common salt, is only trifling, but in pneumonia the salt disappears almost entirely from the urine. A decrease in the alkalinity of the blood has been observed in many cases, as in fevers, uræmia, carbon-monoxide poisoning, diseases of the liver, leucæmia, pernicious anæmia, and diabetes.

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[1] Arch. f. Anat., Physiol. und wissensch. Med., 1876.

[2] Zeitschr. f. physiol. Chem., Bd. 7, S. 22.

[3] Physiol. Chem., 1877–1881, S. 433.

[4] Zeitschr. f. klin. Med., Bd. 11.

The *quantity of glucose* is increased in diabetes (mellitæmia). HOPPE-SEYLER[1] found in one case 9 p. m. glucose in the blood. According to CLAUDE BERNARD,[2] when the quantity of glucose in the blood amounts to 3 p. m. it passes into the urine. The quantity of *urea* is augmented in fevers, also in increased metabolism of proteids. A further increase in the amount of urea in the blood occurs in retarded micturition, as in cholera as well as in cholera infantum (K. MÖRNER[3]), and in affections of the kidneys and the urinary passages. After a ligature of the ureters or after extirpation of the kidneys of animals an accumulation of urea takes place in the blood. In uræmia, ammonia may occur in the blood, which originates from a decomposition of the urea. *Uric acid* is found increased in the blood in gout (GARROD,[4] SALOMON[5]); oxalic acid was also found in the blood in the same disease by GARROD. According to V. JAKSCH fevers alone do not lead to *uricacidæmia.* Uric acid occurs in relatively large quantities, up to 0.08 p. m., in affections of the kidneys, anæmia, and especially such conditions which lead to the symptoms of dyspnœa. Nuclein bases occur sometimes in very small quantities (V. JAKSCH).

Among the *foreign bodies* which are found in the blood the following must be mentioned here: BILIARY ACIDS and BILIARY PIGMENTS (which latter may occur under physiological conditions in a few varieties of blood) in icterus; LEUCIN and TYROSIN in acute atrophy of the liver; ACETON specially in fevers (V. JAKSCH[6]). In melanæmia, especially after continuous malarial fever, black, less often light brown or yellowish, grains of pigment occur in the blood, which, according to the generally received opinion, come from the spleen. After poisoning with potassium chlorate, methæmoglobin is observed in human and in canine blood (MARCHAND[1] and CAHN[2]); but, on the contrary, no formation of methæmoglobin takes place in the blood of rabbits (STOKVIS[3] and KIMMYSER[4]). A formation of methæmoglobin may be caused at the expense of the hæmoglobin by the inhalation of amyl nitrite, as also by the action of a number of other medicinal bodies (HAYEM,[5] DITTRICH,[6] and others).

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[1] Physiol. Chem., S. 430.
[2] Leçons sur le diabète.
[3] See Maly's Jahresber., Bd. 17, S. 453.
[4] Med. Surg. Transactions, Vols. 31 and 37.
[5] Zeitschr. f. physiol. Chem., Bd. 2.
[6] Ueber Acetonurie und Diaceturie. Berlin, 1885.

The *quantity of blood* is indeed somewhat variable in different species of animals and in different conditions of the body; in general we consider the entire quantity of blood in adults as about $\frac{1}{13}$–$\frac{1}{14}$ of the weight of the body, and in new-born infants about $\frac{1}{19}$. Fat individuals are relatively poorer in blood than lean ones. During inanition the quantity of blood decreases less quickly than the weight of the body (PANUM[7]), and it may therefore be also proportionally greater in starving individuals than in well-fed ones.

By careful bleeding the quantity of blood may be considerably diminished without any dangerous symptoms. The loss of blood to $\frac{1}{4}$ of the normal quantity has as sequence no durable sinking of the blood-pressure in the arteries; while the smaller arteries accommodate themselves to the small quantities of blood by contracting (WORM MÜLLER[8]). A loss of blood to $\frac{1}{3}$ of the quantity reduces the blood-pressure considerably, and a loss of $\frac{1}{2}$ of the blood in adults is dangerous to life. The faster the bleeding the more dangerous it is. New-born infants are very sensitive to loss of blood, and likewise fat, old, and weak persons cannot stand much loss of blood. Women can stand loss of blood better than men.

The quantity of blood may be considerably increased by the injection of blood from the same species of animal (PANUM,[9] LANDOIS,[10] WORM MÜLLER,[8] PONFICK[11]). According to WORM

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[1] Virchow's Archiv, Bd. 77, and Arch. f. exp. Path. u. Pharm., Bd. 22.

[2] Arch. f. exp. Path. u. Pharm., Bd. 24.

[3] *Ibid.*, Bd. 21.

[4] Maly's Jahresber., Bd. 14, S. 243.

[5] Comp. rend., Tome 102.

[6] Arch. f. exp. Path. u. Pharm., Bd. 29.

[7] Virchow's Arch., Bd. 29.

[8] Transfusion und Plethora. Christiania, 1875.

[9] Nord. med. Ark., Bd. 7; Virchow's Arch., Bd. 63.

[10] Centralbl. f. d. med. Wissensch., 1875, and Die Transfusion des Blutes. Leipzig, 1875.

[11] Virchow's Arch., Bd. 62.

MÜLLER the normal quantity of blood may indeed be increased to 83% without producing any abnormal conditions or lasting high blood-pressure. An increase of the quantity of blood to 150% may be directly dangerous to life (WORM MÜLLER). If the quantity of blood of an animal is increased by transfusion with blood of the same kind of animal, an abundant formation of lymph takes place. The water in excess is eliminated by the urine; and as the proteid of the blood-serum is quickly decomposed, while the red blood-corpuscles are destroyed much more slowly (TSCHIRJEW,[1] FORSTER,[2] PANUM,[3] WORM MÜLLER[4]), a polycythæmia is gradually produced.

If blood of another kind is transfused, then under certain conditions, according to the quantity of blood introduced, more or less menacing symptoms appear. These appear, for instance, when the blood-corpuscles of the receiver are dissolved easily by the serum of the introduced blood, as, for example, the blood-corpuscles of rabbits on transfusion with a different kind of blood, or the reverse, when the blood-corpuscles of the transfused blood are dissolved by the blood of the receiver; for instance, when the blood of a dog is transfused with rabbit's or lamb's blood, or the blood of a man with lamb's blood (LANDOIS[4]). Before dissolving, the blood-corpuscles may unite in tough agglomerated heaps, which clog up the smaller vessels (LANDOIS). On the other hand, the stromata of the dissolved blood-corpuscles may also give rise to an extensive intravascular coagulation, causing death.

The transfusion should therefore when possible be made with the blood of the same kind of animal, and for the resuscitating action of the blood it is immaterial whether or not it contains the fibrin or the mother-substance of the same. The action of transfused blood depends on its blood-corpuscles, and therefore defibrinated blood acts just like non-defibrinated (PANUM,[4] LANDOIS[5]).

The property of blood-serum of a certain species of animals of dissolving or destroying the blood-corpuscles of another has been called the *globulicidal action* of the serum. According to DAREMBERG,[6] BUCHNER,[7] and others, this property stands in certain relationship to its bactericidal or so-called *microbicidal action*, and these two actions, which have much in common, may be retarded by heating the blood-serum to 55–65° C. The microbicidal action is in part connected with the presence of certain protein bodies acting like enzymes, called *alexins*, and in part to certain mineral bodies such as sodium chloride and alkali. Somewhat similar conditions are also necessary for the globulicidal action. MARAGLIANO[1] has found that the blood-serum in many diseases, such as pneumonia, malaria, typhus, leucmæia, cancerous cachexia, etc., has a destructive action on the red blood-corpuscles. He found the quantity of sodium chloride diminished in such serum, and the globulicidal action was prevented by the addition of NaCl sufficient to make the serum normal in salt.

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[1] Arbeiten aus der physiol. Anstalt zu Leipzig, 1874, S. 292.

[2] Zeitschr. f. Biologie, Bd. 11.

[3] Virchow's Archiv, Bd. 29.

[4] L. c.

[5] L. c.

[6] Sem. médic., 1891, No. 51. Cit. from Maly's Jahresber., Bd. 22.

[7] Arch. f. Hygiene, Bd. 10; Münchener med. Wochenschr., 1892, No. 8, and Berl. klin. Wochenschr., 1892, No. 19.

The quantity of blood in the different organs depends essentially on the activity of the same. During work the exchange of material in an organ is more active than when at rest, and the increased metabolism is connected with a more abundant flow of blood. Although the total quantity of blood in the body remains constant, the distribution of the blood in the various organs may be different at different times. As a rule, the quantity of blood in an organ can be an approximate measure of the more or less active metabolism going on in the same, and from this point of view the distribution of the blood in the different organs and groups of organs is of interest. According to RANKE,[2] to whom we are especially indebted for our knowledge of the relationship of the activity of the organs to the quantity of blood contained therein, of the total quantity of blood (in the rabbit) about ¼ comes to the muscles in rest, ¼ to the heart and the large blood-vessels, ¼ to the liver, and ¼ to the other organs.

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[1] Berl. klin. Wochenschr., 1892, No. 31.

[2] Die Blutvertheilung und der Thätigkeitswechsel der Organe. Leipzig, 1871.

CHAPTER VII.

CHYLE, LYMPH, TRANSUDATIONS AND EXUDATIONS.

I. Chyle and Lymph.

THE lymph is the mediator in the exchange of constituents between the blood and tissues. The bodies necessary for the nutrition of the tissue pass from the blood into the lymph, and the tissues deliver water, salts, and products of metabolism into the lymph. The lymph therefore originates partly from the blood and partly from the tissues. From a purely theoretical standpoint we can, according to HEIDENHAIN, differentiate between blood-lymph and tissue-lymph according to origin. It is impossible at the present time to completely separate what one or the other source delivers; but, thanks to the pioneering investigations of HEIDENHAIN, we have means of exciting a copious flow from one or the other sources of lymph. The action of these means, HEIDENHAIN'S *lymphagogues*, will be closely studied later.

According to older views the lymph was only considered as a filtrate from the blood-fluid. Since the investigations of HEIDENHAIN[1] and HAMBURGER[2] this view cannot be maintained. According to these investigators the lymph is considered under physiological conditions in part as a product of the active, secretory property of the cells of the blood-capillaries.

In chemical respect the lymph is the same as plasma and contains qualitatively the same bodies as this. The most essential difference is of a quantitative nature and consists in that the lymph is poorer in proteids. No essential chemical difference has been found between the lymph and the chyle of starving animals.

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[1] Pflüger's Arch., Bd. 49.

[2] Zeitschr. f. Biologie, Bd. 27, S. 259, and Bd. 30, S. 148; see Ziegler's Beitr. z. pathol. Anat., etc., Bd. 14, S. 448.

After the assimilation of fatty food the chyle differs from the lymph in its wealth of minutely divided fat-globules, which give it a milky appearance; hence the old name "milk-juice."

Chyle and lymph, like the plasma, contain *seralbumin*, *serglobulin*, *fibrinogen*, and *fibrin-ferment*. The two last-mentioned bodies occur only in very small amounts; therefore the chyle and lymph coagulate slowly (but spontaneously) and yield but little fibrin. Like other liquids poor in fibrin-ferment, chyle and lymph do not at once coagulate completely, but repeated coagulations take place.

The extractive bodies seem to be the same as in plasma. *Glucose* is found in about the same quantity as in the blood-serum, but in larger quantities than in the blood; this depends on the fact that the blood-corpuscles contain no glucose. According to RÖHMANN and BIAL[1] lymph contains a diastatic enzyme similar to that in blood-plasma, and LÉPINE[2] has found that the chyle of a digesting dog has great glycolytic activity. DASTRE[3] has studied the glycolytic activity of horse's and cow's lymph, and he finds that it is retarded by the presence of 2 p. m. potassium oxalate. He could also detect glycogen in the cow-lymph which existed in the plasma but not in the form-elements. The amount of *urea* has been determined by WURTZ[4] as 0.12–0.28 p. m. The *mineral bodies* appear to be the same as in plasma.

As form-elements *leucocytes* and *red blood-corpuscles* are common to both chyle and lymph. When it has not left the villi of the intestine chyle contains very few leucocytes, but in the vessels on the peritoneal side of the intestine it is richer in leucocytes. The greatest quantity of leucocytes is found in the chyle between the great mesenteric gland and the cisterna chyli. The chyle is poorer in leucocytes in the thoracic duct, probably because a mixing takes place here with lymph that is poorer in form-constituents from other parts of the body.

Red blood-corpuscles occur in the chyle and lymph in very small quantities. In these liquids, which seem to be free from oxygen, the blood-corpuscles are darker-colored, and only after they have come in contact with the air do they have the light-red color of oxyhæmoglobin and give the surface of the fibrin-clot a beautiful light-red appearance. It has been suggested that this red color originates from the transition forms between red and white blood-corpuscles, in which blood-coloring matters are first formed by the action of the oxygen.

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[1] Pflüger's Archiv, Bdd. 52, 53, and 55.

[2] Compt. rend., Tome 110.

[3] Arch. d. Physiol., Sér. 5, Tome 7.

[4] Compt. rend., Tome 49.

The chyle of starving animals has the appearance of lymph. After partaking of fat or food rich in fat it is milky, and this is partly due to the presence of large fat-globules, as in milk, or partly, and indeed chiefly, the finely divided fat. The nature of the *fats* occurring in the chyle depends on the variety of fat in the food. The disproportionally greater part consists of neutral fats, and even after feeding with abundant amounts of free fatty acids MUNK[1] found in the chyle chiefly neutral fats with a small quantity of fatty acids or soaps.

The *gases* of the chyle have not been studied, and it seems that the gases of an entirely normal human lymph have not thus far been investigated. The gases from dog-lymph contain only traces of oxygen and consist of 37.4–53.1% CO_2 and 1.6% N (AUTHOR[2]) calculated at 0° C. and 760 mm. mercury. The chief mass of the carbon dioxide of the lymph seems to be firmly chemically combined. Comparative analyses of blood and lymph have shown that the lymph contains more carbon dioxide than arterial, but less than venous, blood. The tension of the carbon dioxide of lymph is, according to PFLÜGER and STRASSBURG,[3] smaller than in venous, but greater than in arterial, blood.

The *quantitative composition of the chyle* must naturally be very variable. The analyses thus far made refer only to that mixture of chyle and lymph which is obtained from the thoracic duct. The specific gravity varies between 1.007 and 1.043. As example of the composition of human chyle we will here give two analyses. The first is by OWEN-REES,[4] of the chyle of an executed person, and the second by HOPPE-SEYLER,[5] of the chyle in a case of rupture of the thoracic duct. In the latter case the fibrin had previously separated. The results are in 1000 parts.

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[1] Virchow's Arch., Bdd. 80 and 123.

[2] Die Gase der Hundelymphe. Arbeit. aus d. physiol. Anstalt zu Leipzig, 1871.

[3] Pflüger's Arch., Bd. 6, p. 85.

[4] Cit. from Hoppe-Seyler, Physiol. Chem., S. 595.

[5] *Ibid.*, S. 597.

	No. 1.	No. 2.	
Water	904.8	940.72	water
Solids	95.2	59.28	solids
Fibrin	traces		
Albumin	70.8	36.67	albumin
Fat	9.2	7.23	fat
		2.35	soaps
Remaining organic bodies	10.8	0.83	lecithin
		1.32	cholesterin
		3.63	alcohol extractives
		0.58	water extractives
Salts	4.4	6.80	soluble salts
		0.35	insoluble salts

The quantity of fat is very variable and may be considerably increased by partaking food rich in fats. J. MUNK and A. ROSENSTEIN[1] have investigated the lymph or chyle obtained from a lymph fistula at the end of the upper third of the leg of a girl 18 years old and weighing 60 kg., and the highest quantity of fat in the chylous lymph was 47 p. m. after partaking of fat. In the starvation lymph from the same patient they found only 0.6–2.6 p. m. fat. The quantity of soaps was always small, and on partaking of 41 gm. fat the quantity of soaps was only about $\frac{1}{20}$ of the neutral fats.

A great many analyses of chyle from animals have been made, and they chiefly show the fact that the chyle is a liquid with a very changeable composition which stands closely related to blood-plasma, but with the chief difference that it contains more fat and less solids. The reader is referred to special works for these analyses, as, for example, to v. GORUP-BESANEZ's "Lehrbuch der physiologischen Chemie," 4th edition.

The *composition of the lymph* is also very changeable, and its specific gravity shows about the same variation as the chyle. In the following analyses, 1 and 2, made by GUBLER and QUEVENNE,[2] are the results obtained from lymph from the upper part of the thigh of a woman aged 39; and 3, made by v. SCHERER,[3] is an analysis of lymph from the sac-like dilated lymphatic vessels of the spermatic cord. No. 4 was made by C. SCHMIDT,[4] the data being obtained from lymph from the neck of a colt. The results are in parts per 1000:

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[1] Virchow's Arch., Bd. 123.

[2] Cit. from Hoppe-Seyler's Physiol. Chem., S. 591.

[3] *Ibid.*, S. 591.

[4] L. c.

	1	2	3	4
Water	939.9	934.8	957.6	955.4
Solids	60.1	65.2	42.4	44.6
Fibrin	0.5	0.6	0,4	2.2
Albumin	42.7	42.8	34.7	35.0
Fat, cholesterin, lecithin	3.8	9.2	....	
Extractive bodies	5.7	4.4	....	
Salts	7.3	8.2	7.2	7.5

The salts found by C. SCHMIDT in the lymph of the horse has the following composition, calculated in parts per 1000 parts of the lymph:

Sodium chloride	5.67
Soda	1.27
Potash	0.16
Sulphuric acid	0.09
Phosphoric acid united with alkalies	0.02
Earthy phosphates	0.26

In the cases investigated by MUNK and ROSENSTEIN[1] the quantity of solids in the fasting condition varied between 35.7 and 57.2 p. m. This variation was essentially dependent upon the extent of secretion, so that the low amount coincides with a more active secretion, and the reverse in the other case. The chief portion of the solids consisted of proteids, and the relationship between globulin and albumin was as 1 : 2.4 to 4. The mineral bodies in 1000 parts lymph (chylous) was: NaCl 5.83; Na_2CO_3 2.17; K_2HPO_4 0.28; $Ca_3(PO_4)_2$ 0.28; $Mg_3(PO_4)_2$ 0.09; and $Fe(PO_4)_2$ 0.025.

Under special conditions the lymph may be so rich in finely divided fat that it appears like chyle. Such lymph has been investigated by HENSEN[2] in a case of lymph fistula in a ten-year-old boy, and by LANG[3] in a case of lymph fistula in the left upper part of the thigh of a girl of seventeen. The lymph investigated by HENSEN varied in the quantity of fat, as an average of nineteen analyses, between 2.8 and 36.9 p. m., while that investigated by LANG contained 24.8 p. m. of fat.

The quantity of lymph secreted must naturally change considerably, and we have no means of measuring it. The greatness of the flow of lymph is, as HEIDENHAIN[4] suggests, no measure as to the abundance of supply of nutritive material to the organs, and the lymph-tubes act according to him as "drain-tubes," removing the excess of fluid from the lymph fissures as soon as the pressure therein rises to a certain height. Attempts have been made to determine the quantity of lymph flowing in 24 hours in the thoracic duct of animals. According to HEIDENHAIN the quantity averages 640 c.c. for a dog weighing 10 kilos.

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[1] L. c.

[2] Pflüger's Arch., Bd. 10.

[3] Nord. med. Arkiv., Bd. 16. See Maly's Jahresber., Bd. 4, p. 128.

[4] L. c.

Determinations of the quantity of lymph in man have also been attempted. NOËL-PATON[1] obtained 1 c.c. lymph per minute from the thoracic duct of a patient weighing 60 kilos. The quantity in the 24 hours cannot be calculated from this amount. In the case of MUNK and ROSENSTEIN, 1134–1372 gm. chyle was collected in 12–13 hours after partaking of food. In the fasting condition or after starving for 18 hours they found 50 to 70 gm. per hour, sometimes 120 gm. and above, especially in the first few hours after powerful muscular exercise.

Several circumstances have a marked influence on the extent of lymph secretion. During starvation less lymph is secreted than after partaking of food. NASSE[2] has observed in dogs that the formation of lymph is increased 36% more after feeding with meat than after feeding with potatoes, and about 54% more than after 24 hours' deprivation of food.

An increase in the total blood-pressure, as by transfusion of blood, also especially on preventing the flow of blood by means of ligatures, causes an increase in the quantity of lymph. According to HEIDENHAIN,[3] on the contrary, a very considerable change in the pressure in the aorta causes only a little change in the abundance of the lymph-flow. The quantity of lymph may be raised by powerful active and passive movements of the limbs (LESSER[4]). Under the action of curara an increase of the lymph-secretion is observed (PASCHUTIN,[5] LESSER), and the quantity of solids in the lymph is also increased.

The means of inciting the lymph-flow are of special interest. They are called *lymphagogues*, and according to HEIDENHAIN they are of two kinds. The lymphagogues of the first series are still unknown bodies which may be extracted by water from the muscles of the crab, the head and body of the blood- and horse-leech, the body of anodons, the intestine and liver of dogs. Peptone (HEIDENHAIN,[1] STARLING[2]) and sometimes egg-albumin may act as a lymphagogue of this series. These bodies when injected into the blood in watery solution cause an increase in the secretion of lymph, and the quantity of organic substances in the lymph is increased at the same time, while the amount of salts remains unchanged. The blood becomes more concentrated, due to extravasation of plasma, the remaining plasma less concentrated, that is, poorer in proteids. These lymphagogues produce chiefly blood-lymph, and their action is not influenced by any change in the blood-pressure. As further the composition of the lymph and blood-plasma may be changed by membrane filtration under an increased pressure, in which the lymph becomes richer and the blood-plasma poorer in proteids, still the increase in the lymph formation cannot be explained, according to HEIDENHAIN, by the mechanical filtration process. According to him the capillary cells must take an active secretory part in this secretion.

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[1] Journal of Physiol., Vol. 11.

[2] Cit. from Hoppe-Seyler's Physiol. Chem., S. 593.

[3] L. c.

[4] Arbeiten aus der physiol. Anstalt zu Leipzig, Jahrg. 6, S. 94.

[5] *Ibid.*, Jahrg. 7, S. 216.

The lymphagogues of the second series are crystalline substances, such as sugar, urea, sodium chloride, and other salts. These bodies when injected into the blood cause a very copious secretion of lymph, but thereby the blood as well as the lymph becomes richer in water and poorer in solids. This increase in quantity of water in the lymph and blood, which causes an abundant excretion of urine, can only be attributed to a more copious supply of water of the tissue-elements, and the lymph secreted under these circumstances is not blood-lymph but chiefly tissue-lymph. These bodies inciting the lymph-flow pass from the blood into the lymph-spaces by diffusion and also in part (at least in the case of sugar) by the secretory activity of the capillary walls, and have an attraction for the tissue-water of the cells, fibres, etc. This water passes in part by diffusion into the blood and then into the urine, and another part flows into the lymph-canals.

HEIDENHAIN has observed that if the arterial blood-pressure is reduced to zero or near thereto, the lymph-current may nevertheless continue for one or two hours, and he also found that a change in the aorta-pressure of between 10–20 mm. on one side and 150–200 mm. on the other had only little influence on the extent of lymph-flow. These facts, as also the action of the bodies exciting the lymph-flow, do not, according to HEIDENHAIN, agree with the ordinary view that the lymph is only a filtrate or diffusate of the blood. According to this author, we must also consider that the cells of the capillary walls are directly concerned in a secretory way in the lymph-formation.

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[1] L. c.

[2] Journal of Physiol., Vol. 14.

HAMBURGER[1] has arrived at a similar view, independently of HEIDENHAIN, as to the importance of the capillary endothelium in the lymph-formation.

STARLING[2] has lately suggested a series of experiments in opposition to HEIDENHAIN'S view, in which he comes to the conclusion that the lymph-formation is dependent upon two factors, namely, the permeability of the vascular walls and the blood-pressure, and he explains the action of the bodies exciting a lymph-flow in a different way from HEIDENHAIN. The recent researches of STARLING and LEATHES,[3] ORLOW,[4] and COHNSTEIN[5] on the absorption from serous cavities and on the formation of transudations strongly emphasize the importance of osmosis and filtration for absorption and formation of transudations or lymph.

The lymphagogues of the first series, according to STARLING, cause such an abundant lymph-flow in the liver that the entire increase in the lymph-current is, in this case, due to the formation of liver-lymph. This lymph is very rich in solids, and the great concentration of the lymph discharged under these conditions is due to this fact. The blood-plasma becomes poorer in solids, partly due to the abundant formation of concentrated liver-lymph and partly by admixture with lymph from other parts of the body which is poor in solids. The variations found by HEIDENHAIN in the concentration of the lymph and blood-plasma is, according to STARLING, no proof as to a special secretory activity of the capillary endothelium. The abundant secretion of concentrated liver-lymph cannot be explained by a variation in the blood-pressure, and according to STARLING it is due essentially to an increased permeability of the liver-capillaries. The action of these lymphagogues on the cells is not, according to him, a physiological one, exciting secretion, but a pathological and toxic one which increases the permeability of the capillary walls.

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[1] See Hamburger, Zeitschr. f. Biologie, Bd. 27, S. 259, and Bd. 30, S. 143. Also Hamburger, Hydrops von mikrobiellem Ursprung, in Beitr. zur path. Anat. und zur allg. Pathol., Bd. 14, S. 443.

[2] Journal of Physiol., Vols. 16 and 17.

[3] Journ. of Physiol., Vol. 18.

[4] Pflüger's Arch., Bd. 59.

[5] Virchow's Arch., Bd. 135, and Pflüger's Arch., Bd. 59.

The lymphagogues of the second series act, according to STARLING, first by osmosis, causing an abundant flow of water into the blood and thereby increasing the pressure in the capillaries, producing a stronger filtration. The more abundant current of lymph in the thoracic duct is caused in this case by a greater pressure in the abdominal capillaries.

II. Transudations and Exudations.

The serous membranes are normally kept moistened by liquids whose quantity is only sufficient in a few instances, as in the pericardial cavity and the subarachnoidal space, for a complete chemical analysis to be made of them. Under diseased conditions an abundant transudation may take place from the blood into the serous cavities, into the subcutaneous tissues, or under the epidermis; and in this way pathological transudations are formed. Such true transudations, which are similar to lymph, are generally poor in form-elements and leucocytes, and yield only very little or almost no fibrin, while the inflammatory transudations, the so-called exudations, are generally rich in leucocytes and yield proportionally more fibrin. As a rule, the richer a transudation is in leucocytes the closer it stands to pus, while when it has a diminished quantity of leucocytes it is more nearly like real transudations or lymph.

It is ordinarily accepted that filtration is of the greatest importance in the formation of transudations and exudations. The facts coincide with this view, namely, that all these fluids contain the salts and extractive bodies occurring in the blood-plasma in about the same quantity as the blood-plasma, while the amount of proteids is habitually smaller. While the different fluids belonging to this group have about the same quantities of salts and extractive bodies, they differ from each other chiefly in containing differing quantities of proteid and form-elements, as well as varying quantities of transformation and decomposition products of these latter—changed blood-corloring matters, cholesterin, etc., etc.

It must be apparent that the circulation and pressure conditions must have an essential influence on the quantity and composition of the transudations, but their action has been little studied. An increase in the vein-pressure causes, according to SENATOR,[1] an increase in the quantity of transudation and the quantity of proteid contained, while the amount of salts does not markedly change. Nothing positive is known in regard to the variations in the quantity of proteid by simple arterial hyperæmia.

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[1] Virchow's Arch., Bd. 111.

The process, as suggested by COHNHEIM,[1] of the changed permeability of the capillary walls in disease is a second important factor in the formation of transudations. The circumstance that the greatest quantity of proteid occurs in transudations in inflammatory processes, to which is also due the abundant quantity of form-elements in such transudations, has been explained by this hypothesis. The greater quantity of proteid in the transudations in formative irritation is in great part explained by the large amount of destroyed form-elements. The interesting observation made by PALJKULL,[2] that in such cases in which an inflammatory irritation has taken place the fluid contains nucleoalbumin (or nucleoproteids ?), while these substances do not occur in transudations in the absence of inflammatory processes, can be explained by the presence of form-elements.

As the secretory importance of the capillary endothelium has been made probable by the investigations of HEIDENHAIN and HAMBURGER, it is *a priori* to be expected that an abnormal increased secretory activity of the endothelium is a third cause of transudations. Certain observations of HAMBURGER in a case of dropsy,[3] in which the transudation was probably produced by the lymph-exciting action of a metabolic product formed by a bacterium, speak for the correctness of this assumption. HAMBURGER therefore considers the irritation of the endothelium of the capillaries by means of a special substance exciting lymph-flow and formed in disease as a third cause of the transudations. The question whether this substance acts secretory in HEIDENHAIN's sense or increases the permeability in STARLING's sense must be proved.

That the conditions of the blood-capillaries in the different vascular regions have an effect on the quantity of proteid has been partly explained by the varying secretory activity of the capillary endothelium (C. SCHMIDT[4]). For example, the amount of proteid in the PERICARDIAL, PLEURAL, and PERITONEAL FLUIDS is considerably greater than in those fluids which are found in the SUBARACHNOIDAL SPACE, in the SUBCUTANEOUS TISSUES, or in the AQUEOUS HUMOR, which are poor in proteid. The condition of the blood also greatly affects the transudations, for in hydræmia the amount of proteid in the transudation is very small. With the increase of the age of a transudation, of a hydrocele fluid for instance, the quantity of proteid is increased, probably by resorption of water, and indeed exceptional cases may occur in which the amount of proteid, without any previous hemorrhage, is even greater than in the blood-serum.

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[1] Cohnheim, Vorlesungen über allg. Path., 2. Aufl., Part 1.

[2] Upsala Läkarefs. Förhandl., Bd. 27, and Maly's Jahresber., Bd. 22.

[3] See Ziegler's Beiträge, Bd. 14.

[4] Cit. from Hoppe-Seyler's Physiol. Chem., p. 607.

The proteids of transudations are chiefly seralbumin, serglobulin, and a little fibrinogen. The non-inflammatory transudations do not as a rule coagulate spontaneously, or very slowly. On the addition of blood or blood-serum they coagulate. Inflammatory exudations coagulate spontaneously. PAIJKULL[1] has shown that these often contain nucleoalbumin. Mucoid substances, which were first observed by the AUTHOR[2] in a few cases of ascitic fluid, without complication with ovarial tumors, seem, according to PAIJKULL, to be regular constituents of transudations. The relationship between globulin and seralbumin varies very much in different cases, but, as HOFFMANN[3] and PIGEAUD[4] have shown, the variation is in each case the same as the blood-serum of the individual.

The specific gravity runs rather parallel with the quantity of proteid. The varying specific gravity has been suggested as a means of differentiation between transudations and exudations by REUSS,[5] as the first often show a specific gravity below 1015–1010, while the others have a specific gravity of 1018 or above. This rule holds good in many but not in all cases.

The *gases* of the transudations consist of carbon dioxide besides small amounts of nitrogen and traces of oxygen. The tension of the carbon dioxide is greater in the transudations than in the blood. On mixing with pus the amount of carbon dioxide is decreased.

The *extractives* are, as above stated, the same as in the blood-plasma; but sometimes extractive bodies occur, such as allantoin in dropsical fluids (MOSCATELLI[1]), which have not been detected in the blood. *Urea* seems to occur in very variable amounts. *Glucose*, or at least a fermentable substance which reduces copper oxide in alkaline liquids, occurs in most transudations. *Succinic acid* has been found in a few cases in hydrocele fluids, while in other cases it is entirely absent. *Leucin* and *tyrosin* have been found in transudations from diseased livers and in pus-like transudations which have undergone decomposition. Among other extractives found in transudations we must mention *uric acid*, *allantoin*, *xanthin*, *creatin*, *inosit*, and *pyrocatechin*.

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[1] L.c.

[2] Zeitschr. f. physiol. chem., Bd. 15.

[3] Arch. f. exp. Path. u. Pharm., Bd. 16.

[4] See Maly's Jahresber., Bd. 16.

[5] Deutsch. Arch. f. klin. Med., Bd. 28.

As above stated, irrespective of the varying number of form-elements contained in the different transudations, the quantity of proteid is the most characteristic chemical distinction in the composition of the various transudations; therefore a quantitative analysis is only of importance in so far as it considers the quantity of proteid. On this account the following quantitative composition is referred to the chief weight, the quantity of proteid.

Pericardial Fluid. The quantity of this fluid is also, under certain physiological conditions, so large that a sufficient quantity for chemical investigation was obtained from a person who had been executed. This fluid is lemon-yellow in color, somewhat sticky, and yields more *fibrin* than other transudations. The amount of solids, according to the analyses performed by v. GORUP-BESANEZ,[2] WACHSMUTH,[3] and HOPPE-SEYLER,[4] is 37.5–44.9 p. m., and the amount of proteid is 22.8–24.7 p. m. The analysis made by the AUTHOR of a fresh pericardial fluid from a young man who had been executed yielded the following results, calculated in 1000 parts by weight:

Water	960.85		
Solids	39.15		
Proteids	28.60	Fibrin	0.31
		Globulin	5.95
		Albumin	22.34
Soluble salts	8.60	NaCl	7.28
Insoluble salts	0.15		
Extractive bodies	2.00		

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[1] Zeitschr. f. physiol. Chem., Bd. 13.

[2] v. Gorup-Besanez, Lehrbuch d. physiol. Chem., 4. Aufl., S. 401.

[3] Virchow's Arch., Bd. 7.

[4] Physiol. Chem., S. 605.

FRIEND[1] has found nearly the same composition for a pericardial fluid from a horse, with the exception that this liquid was relatively richer in globulin. The ordinary statement that pericardial fluids are richer in fibrinogen than other transudations is hardly based on sufficient proof. In a case of chylopericardium, which was probably due to the rupture of a chylus vessel or caused by a capillary exudation of chyle because of stoppage, HASEBROEK[2] found in 1000 parts of the analyzed fluid 103.61 parts solids, 73.79 albuminous bodies, 10.77 fat, 3.34 cholesterin, 1.77 lecithin, and 9.34 salts.

The **pleural fluid** occurs under physiological conditions in such small quantities that a chemical analysis of the same cannot be made. Under pathological conditions this fluid may show very variable properties. In a few cases it is nearly serous, in others again sero-fibrinous, and in others similar to pus. There is a corresponding variation in the specific gravity and the properties in general. If a pus-like exudation is kept closed for a long time in the pleural cavity, a more or less complete maceration and solution of the pus-corpuscles is found to take place. The ejected yellowish-brown or greenish fluid may then be as rich in solids as the blood-serum; and an abundant flocculent precipitate of a nucleoalbumin (the *pyin* of early writers) may be obtained on the addition of acetic acid. This precipitate is soluble with difficulty in an excess of acetic acid.

Numerous analyses, by many investigators,[3] of the quantitative composition of pleural fluids under pathological conditions are at hand. From these analyses we learn that in hydrothorax the specific gravity is lower and the quantity of proteid less than in pleuritis. In the first case the specific gravity is generally less than 1015, and the quantity of proteid 10–30 p. m. In acute pleuritis the specific gravity is generally higher than 1020, and the quantity of proteid 30–65 p. m. The quantity of fibrinogen, which in hydrothorax is about 0.1 p. m., may amount to more than 1 p. m. in pleuritis. In pleurisy with an abundant gathering of pus the specific gravity may rise even to 1030, according to the observations of the AUTHOR. The quantity of solids is often 60–70 p. m., and may be even more than 90–100 p. m. (AUTHOR). Mucoid substances have also been detected in pleural fluids by PAIJKULL. Cases of chylous pleurisy are also known; in such a case MÉHU[1] found 17.93 p. m. fat and cholesterin in the fluid.

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[1] Halliburton: Text-book of Chem. Physiol., etc. London, 1891. P. 347.

[2] Zeitschr. f. physiol. chem., Bd. 12.

[3] See the works of Méhu, Runeberg, F. Hoffmann, Reuss, Neuenkirchen, all of which are cited in Bernheim's paper in Virchow's Arch., Bd. 131, S. 274. See also Paijkull, l. c., and Halliburton's Text-book, p. 346.

The quantity of **peritoneal fluid** is very small under physiological conditions. The investigations refer only to the fluid under diseased conditions (*dropsical* or *ascitic fluid*). The color, transparency, and consistency of these may vary greatly.

In cachectic conditions or a hydræmic condition of the blood the fluid has little color, is milky, opalescent, watery, does not coagulate spontaneously, has a very low specific gravity, 1005–1010–1015, and is nearly free from form-elements.

The ascitic fluid in portal stagnation, or generally in venous stagnation, has a low specific gravity and ordinarily less than 20 p. m. proteid, although in certain cases the quantity of proteid may rise to 35 p. m. In carcinomatous peritonitis it may have a cloudy, dirty-gray appearance, due to its richness in form-elements of various kinds. The specific gravity is then higher, the quantity of solids greater, and it often coagulates spontaneously. In inflammatory processes it is straw- or lemon-yellow in color, somewhat cloudy or reddish, due to leucocytes and red blood-corpuscles, and from great richness in leucocytes it may appear more like pus. It coagulates spontaneously, and may be relatively richer in solids. It contains regularly 30 p. m. or more proteid (although exceptions with less proteid occur), and may have a specific gravity of 1.030 or above. By rupture of a chylous vessel the dropsical fluid may be rich in very finely emulsified fat (CHYLOUS ASCITES). In such cases 3.86–10.30 p. m. fat has been found in the dropsical fluid (GUINOCHET,[2] HAY[3]), or even 17–43 p. m. fat has been found by MINKOWSKY. By admixture of this fluid with the fluid from an ovarian cyst it may sometimes contain pseudomucin (see Chapter XIII). We also have cases in which the ascitical fluid contains mucoids which may be precipitated by alcohol after removal of the proteids by coagulation at boiling temperature. Such substances, which yield a reducible substance on boiling with acids, have been found by the author in tuberculous peritonitis and in cirrhosis hepatis syphilitica in men. According to the investigations of PAIJKULL[1] these substances seem to occur often and perhaps habitually in the ascitic fluids.

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[1] Arch. gén. de med., 1886, Tome 2. Cit from Maly's Jahresber., Bd. 16.

[2] See Straus, Arch. de physiol, Tome 18. Cit. from Maly's Jahresber., Bd. 17.

[3] See Maly's Jahresber., Bd. 16, S. 475.

As the quantity of proteid in ascitic fluids is dependent upon the same circumstances as in other transudations and exudations, it is sufficient to give the following example of the composition, taken from BERNHEIM'S[2] treatise. The results are expressed in 1000 parts of the fluid:

	Max.	Min.	Mean.
Cirrhosis of the liver	34.5	5.6	9.69 — 21.06
Bright's disease	16.11	10.10	5.6 10.36
Tuberculous and idiopathic peritonitis	55.8	18.72	30.7 — 37.95
Carcinomatous peritonitis	54.20	27.00	35.1 — 58.96

Urea has also been found in ascitical fluids, sometimes only as traces, sometimes in larger quantities (4 p. m. in albuminuria), also *uric acid*, *allantoin* in cirrhosis of the liver (MOSCATELLI[3]), *xanthin*, *creatin*, *cholesterin*, and *glucose*.

Hydrocele and Spermatocele Fluids. These fluids differ from each other in various ways. The hydrocele fluids are generally colored light or darker yellow, sometimes brownish with a shade of green. They have a relatively higher specific gravity, 1.016–1.026, with a variable but generally higher amount of solids, an average of 60 p. m. They sometimes coagulate spontaneously, sometimes only after the addition of fibrin-ferment or blood. They contain *leucocytes* as chief form-elements. Sometimes they contain smaller or larger amounts of *cholesterin crystals*.

The spermatocele fluids, on the contrary, are as a rule colorless, thin, cloudy like water mixed with milk. They sometimes have an acid reaction. They have a lower specific gravity, 1.006–1.010, a lower amount of solids—an average of about 13 p. m.,—and do not coagulate either spontaneously or after the addition of blood. They are, as a rule, poor in proteid and contain *spermatozoa*, *cell-detritus*, and *fat-globules* as form-constituents. To show the unequal composition of these two kinds of fluids we will give the average results (calculated in parts per 1000 parts of the fluid) of 17 analyses of hydrocele fluids and 4 of spermatocele fluids made by the author:[4]

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[1] L. c.

[2] L. c. As it was impossible to derive mean figures from those given by Bernheim, the author has given above the maximum and minimum of the averages given by him.

[3] L. c.

[4] Upsala Läkaref. Förh., Bd. 14, and Maly's Jahresber., Bd. 8, S. 347.

	Hydrocele.	Spermatocele.
Water	938.85	986.33
Solids	61.15	13.17
Fibrin	0.59	....
Globulin	13.25	0.59
Seralbumin	35.94	1.82
Ether extractive bodies	4.02	10.76
Soluble salts	8.60	
Insoluble salts	0.66	

In the hydrocele fluid traces of *urea* and a reducing substance have been found, and in a few cases also *succinic acid* and *inosit*. A hydrocele fluid may, according to DEVILLARD,[1] sometimes contain paralbumin or metalbumin (?). Cases of chylous hydrocele are also known.

Cerebro-spinal Fluid. This fluid has heretofore been considered as a secretion and not a transudation. But as we now consider not only the lymph as part secretion, but also the transudations, such a difference between this fluid and the others cannot be maintained. The cerebro-spinal fluid is thin, water-clear, of low specific gravity, 1007–1008. The spina bifida fluid is very poor in solids, 8–10 p. m., with only 0.19–1.6 p. m. proteid. The fluid of chronic hydrocephalus is somewhat richer in solids (13–19 p. m.) and proteids. According to HALLIBURTON[2] the proteid of the cerebro-spinal fluid is a mixture of *globulin* and *albumoses*; occasionally some peptone occurs, and more rarely, in special cases, seralbumin appears. An optically inactive, non-fermentable, reducing substance, seemingly *pyrocatechin* (HALLIBURTON), has been observed in this fluid. The older statement that the cerebro-spinal fluid differs from the other transudations in a greater wealth of potassium salts has not been confirmed by recent investigations of YVON[3] and HALLIBURTON. According to CAVAZZANI[4] the cerebro-spinal fluid is more alkaline and richer in solids in the morning than in the evening.

Aqueous Humor. This fluid is clear, alkaline, and has a specific gravity of 1.003–1.009. The amount of solids is on an average 13 p. m., and the amount of proteids only 0.8–1.2 p. m. The proteid consists of *seralbumin* and *globulin* and very little *fibrinogen*. According to GRUENHAGEN,[5] it contains *paralactic acid*, another dextrogyrate substance, and a *reducing body* which is not similar to glucose or dextrin. PAUTZ[1] found urea and sugar in the aqueous humor of oxen.

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[1] Bull. soc. chim., Tome 49, p. 617.

[2] Halliburton's Text-book, pp. 355–361.

[3] Journ. de Pharm. et de Chim. (4 Sér.), Tome **26**.

[4] Maly's Jahresber., Bd. 22, S. 346.

[5] Pflüger's Arch., Bd. 43.

Blister-fluid. The content of blisters caused by burns, and of vesicator blisters and the blisters of the *pemphigus chronicus*, is generally a fluid rich in solids and proteids (40–65 p. m.). This is especially true of the contents of vesicatory blisters, which also contain a substance that *reduces* copper oxide. The fluid of the pemphigus is slimy and alkaline in reaction.

The fluid of **subcutaneous œdema**. This is, as a rule, very poor in solids, purely serous, does not contain fibrinogen, and has a specific gravity of 1.005–1.010. The quantity of proteids is in most cases lower than 10 p. m.,—according to HOFFMANN 1–8 p. m.,—and in serious affections of the kidneys, generally with amyloid degeneration, less than 1 p. m. has been shown (HOFFMANN[2]). The œdema fluid also habitually contains *urea*, 1–2 p. m., and also a *reducing substance*.

The FLUID OF THE TAPEWORM cyst is related to the transudations. It is thin and colorless, and has a specific gravity of 1.005–1.015. The quantity of solids is 14–20 p. m. The chemical constituents are *glucose* (2.5 p. m.), *inosit*, traces of *urea*, *creatin*, *succinic acid*, and salts (8.3–9.7 p. m.). Proteids are only found in traces, and then only after an inflammatory irritation. In the last-mentioned case 7 p. m. proteids have been found in the fluid.

The Synovial Fluid and Fluid in Synovial Cavities around Joints, etc. The synovia is hardly a transudation, but it is often treated as an appendix to the transudations.

The synovia is an alkaline, sticky, fibrous, yellowish fluid which is cloudy, from the presence of cell-nuclei and remains of destroyed cells, but is sometimes clear. It contains also, besides *proteids* and salts, a substance similar to *mucin* in physical properties. The nature of these mucin-like constituents of physiological synovial fluids has not been determined. The author[3] has found a mucin-like substance in pathological synovial fluid, but it was not true mucin. It acts like a nucleoalbumin or a nucleoproteid, and gave no reducing substance when boiled with acid. SALKOWSKI[4] also found a mucin-like substance in a pathological synovial fluid, which was neither mucin nor nucleoalbumin. He called the substance "*synovin*."

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[1] Zeitschr. f. Biologie, Bd. 31.

[2] Deutsch. Arch. f. klin. Med., Bd. 44.

[3] Upsala Läkaref. Förhandl., Bd. 17.

[4] Virchow's Arch., Bd. 131.

The composition of synovia is not constant, but varies in rest and in motion. In the last-mentioned case the quantity of fluid is less, but the amount of the mucin-like body, proteids, and of the extractive bodies is greater, while the quantity of salts is diminished. This may be seen from the following analyses by FRERICHS.[1] The figures represent parts per 1000.

	I. Synovia from a Stall-fed Ox.	II. Synovia from a Field-fed Ox.
Water	969.9	948.5
Solids	30.1	51.5
Mucin-like body	2.4	5.6
Proteids and extractives	15.7	35.1
Fat	0.6	0.7
Salts	11.3	9.9

The synovia of new-born babes corresponds to that of resting animals. The fluid of the bursæ mucosæ, as also the fluid in the synovial cavities around joints, etc., is similar to synovia from a qualitative standpoint.

III. Pus.

Pus is a yellowish-gray or yellowish-green, creamy mass of a faint odor and an unsavory, sweetish taste. It consists of a fluid, the *pus-serum*, in which solid particles, the *pus-cells*, swim. The number of these cells varies so considerably that the pus may at one time be thin and at another time so thick that it scarcely contains a drop of serum. The specific gravity, therefore, may also greatly vary, namely, between 1.020 and 1.040, but ordinarily it is 1.031–1.033. The reaction of fresh pus is generally alkaline, but it may become neutral or acid from a decomposition in which fatty acids, glycero-phosphoric acid, and also lactic acid are formed. It may become strongly alkaline when putrefaction occurs with the formation of ammonia.

In the chemical investigation of pus the pus-serum and the pus-corpuscles must be studied separately.

Pus-serum. Pus does not coagulate spontaneously nor after the addition of defibrinated blood. The fluid in which the pus-corpuscles are suspended is not to be compared with the plasma, but rather with the serum. The pus-serum is pale yellow, yellowish green, or brownish yellow, and has an alkaline reaction. It contains, for the most part, the same constituents as the blood-serum; but sometimes besides these—when, for instance, the pus has remained in the body for a long time—it contains a nucleoalbumin or nucleoproteid which is precipitated by acetic acid and soluble with great difficulty in an excess of the acid (*pyin* of the older authors). This nucleoalbumin seems to be formed from the hyaline substance of the pus-cells by maceration. The pus-serum contains, moreover, at least in many cases, no fibrin-ferment. According to the analyses of Hoppe-Seyler,[1] the pus-serum contains in 1000 parts:

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[1] Wagner's Handwörterbuch, Bd. 3, Abth. 1, S. 463.

	I.	II.
Water	913.7	905.65
Solids	86 3	94.35
Proteids	63.23	77.21
Lecithin	1.50	0.56
Fat	0.26	0.29
Cholesterin	0.53	0.87
Alcohol extractives	1.52	0.73
Water extractives	11.53	6.92
Inorganic salts	7.73	7.77

The ash of pus-serum has the following composition, calculated to 1000 parts of the serum:

	I.	II.
NaCl	5.22	5.39
Na_2SO_4	0.40	0.31
Na_2HPO_4	0.98	0.46
Na_2CO_3	0.49	1.13
$Ca_3(PO_4)_2$	0.49	0.31
$Mg_3(PO_4)_2$	0.19	0.12
PO_4 (in excess)	....	.05

The **pus-corpuscles** are generally thought to consist in great part of emigrated white blood-corpuscles (emigration hypothesis), and their chemical properties have therefore been given above. We consider the molecular grains, fat-globules, and red blood-corpuscles rather as casual form-elements.

The pus-cells may be separated from the serum by centrifugal force, or by decantation directly or after dilution with a solution of sodium sulphate in water (1 vol. saturated sodium-sulphate solution and 9 vols. water), and then washed by this same solution in the same manner as the blood-corpuscles.

The chief constituents of the pus-corpuscles are albuminous bodies of which the largest proportion seems to be a nucleoproteid which is insoluble in water and which expands into a tough, slimy mass when treated with a 10% common-salt solution. This proteid substance, which is soluble in alkali but quickly changed thereby, is called ROVIDAS's *hyaline substance*, and the property of the pus of being converted into a slime-like mass by a solution of common salt depends on this substance. Besides this substance we find in the pus-cells also an albuminous body which coagulates at 48–49° C., as well as *serglobulin* (?), *seralbumin*, a substance similar to coagulated albumin (MIESCHER),[1] and lastly *peptone* (HOFMEISTER).[2]

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[1] Med. chem. Untersuch., S. 490.

We also find in the protoplasm of the pus-cells, besides the proteids, *lecithin*, *cholesterin*, *xanthin bodies*, *fat*, and *soaps*. HOPPE-SEYLER has found *cerebrin*, a decomposition product of a protagon-like substance, in pus (see Chapter XII). KOSSEL and FREYTAG[3] have isolated from pus two substances, *pyosin* and *pyogenin*, which belong to the cerebrin group (see Chapter XII). HOPPE-SEYLER[4] claims that *glycogen* appears only in the living, contractile white blood-cells and not in the dead pus-corpuscles. SALOMON[5] has nevertheless found glycogen in pus. The cell-nucleus contains *nuclein* and nucleoproteids.

The *mineral constituents* of the pus-corpuscles are potassium, sodium, calcium, magnesium, and iron. A part of the alkalies is found as chlorides, and the remainder, as well as the other bases, exists as phosphates.

The quantitative composition of the pus-cells from the analyses of HOPPE-SEYLER is as follows, in parts per 1000 of the dried substance:

	I.		II.
Proteids	137.62	685.85	673.69
Nuclein	342.57		
Insoluble bodies	205.66		
Lecithin	143.83		75.64
Fat			75.00
Cholesterin	74.00		72.83
Cerebrin	51.99		102.84
Extractive bodies	44.33		

MINERAL SUBSTANCES IN 1000 PARTS OF THE DRIED SUBSTANCE.

NaCl	4.35
$Ca_3(PO_4)_2$	2.05
$Mg_3(PO_4)_2$	1.13
$FePO_4$	1.06
PO_4	9.16
Na	0.68
K	traces (?)

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[1] Hoppe-Seyler's Med. chem. Untersuch., S. 441.

[2] Zeitschr. f. physiol. Chem., Bd. 4.

[3] *Ibid.*, Bd. 17, S. 452.

[4] Physiol. Chem., S. 790.

[5] Deutsch. med. Wochenschr., 1877, No. 8.

MIESCHER has obtained other results for the alkali combinations, namely: potassium phosphate 12, sodium phosphate 6.1, earthy phosphate and iron phosphate 4.2, sodium chloride 1.4, and phosphoric acid combined with organic substances 3.14–2.08 p. m.

In pus from congested abscesses which have stagnated for some time we find *peptone*, *leucin*, and *tyrosin*, free *fatty acids*, and *volatile fatty acids*, such as formic acid, butyric acid, valerianic acid. We also sometimes find *chondrin* (?) and *glutin* (?), *urea*, *glucose* (in diabetes), *bile-pigments* and *bile-acids* (in catarrhal icterus).

As more specific but not constant constituents of the pus we must mention the following: *pyin*, which seems to be a nucleoalbumin or nucleoproteid precipitable by acetic acid, and also *pyinic acid* and *chlorrhodinic acid*, which have been so little studied that they cannot be more fully treated here.

In many cases a blue, more rarely a green, color has been observed in the pus. This depends on the presence of a variety of vibrios (LÜCKE) from which FORDOS[1] and LÜCKE[2] have isolated a crystallizable coloring matter partly blue and partly yellow, *pyocyanin* and *pyoxanthose*.

Appendix.

Lymphatic Glands, Spleen, etc.

The **Lymphatic Glands.** The cells of the lymphatic glands are found to contain the protein substances occurring generally in cells (Chapter V, p. 90–91). Albumoses and peptones may also occur as products of a post-mortem decomposition. Besides the other ordinary tissue-constituents, such as collagen, reticulin, elastin, and nuclein, we find in the lymphatic glands also *cholesterin*, *fat*, *glycogen*, *sarcolactic acid*, *xanthin bodies*, and *leucin*. In the inguinal glands of an old woman OIDTMANN[3] found 714.32 p. m. water, 284.5 p. m. organic and 1.16 p. m. inorganic substances.

The Spleen. The pulp of the spleen cannot be freed from blood. The mass which is separated from the spleen capsule and the structural tissue by pressure and which ordinarily serves as material for chemical investigations is therefore a mixture of blood and spleen constituents. For this reason the albuminous bodies of the spleen are little known. As characteristic constituents we have *albuminates containing iron*, and especially a protein substance which does not coagulate on boiling, and which is precipitated by acetic acid and yields an ash containing much phosphoric acid and iron oxide.[1]

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[1] Compt. rend., Tome 51 and 56.

[2] Arch. f. klin. Chirurg., Bd. 3.

[3] v. Gorup-Besanez, Lehrbuch, 4. Aufl., S. 732.

The pulp of the spleen, when fresh, has an alkaline reaction, but quickly turns acids, due partly to the formation of free *paralactic acid* and partly perhaps to *glycero-phosphoric acid.* Besides these two acids there have been found in the spleen also *volatile fatty acids*, as formic, acetic, and butyric acids, as well as *succinic acid*, *neutral fats*, *cholesterin*, traces of *leucin*, *inosit* (in ox-spleen), *scyllit*, a body related to inosit (in the spleen of plagiostoma), *glycogen* (in dog-spleen), *uric acid*, *xanthin bodies*, and *jecorin* (BALDI[2]).

Among the constituents of the spleen the *deposit rich in iron*, which consists of ferruginous granules or conglomerate masses of them, and closely studied by NASSE, is of special interest. These iron grains produced by the transformation of the red corpuscles, and which also occur in old thrombi, are chiefly produced when stagnant blood-corpuscles are not dissolved, and they may be formed either extracellular or intracellular when the blood-corpuscles are taken up by the colorless cells. This deposit does not occur to the same extent in the spleen of all animals. It is found especially abundant in the spleen of the horse. NASSE[3] on analyzing the grains (from the spleen of a horse) obtained 840–630 p. m. organic and 160–370 p. m. inorganic substances. These last consisted of 566–726 p. m. Fe_2O_3, 205–388 p. m. P_2O_5, and 57 p. m. earths. The organic substances consisted chiefly of proteids (660–800 p. m.), nuclein, 52 p. m. (maximum), a yellow coloring matter, extractive bodies, fat, cholesterin, and lecithin.

In regard to the *mineral constituents* it is to be observed that the amount of iron in adults is strikingly large, and further that the amount of sodium and phosphoric acid is smaller than that of potassium and chlorine. The amount of iron in new-born and young animals is small (LAPICQUE,[4] KRÜGER, and PERNOU[5]), in adults more appreciable, and in old animals sometimes very considerable. NASSE[1] found nearly 50 p. m. iron in the dried pulp of the spleen of an old horse.

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[1] v. Gorup-Besanez, Lehrbuch, 4. Aufl., S. 717.

[2] Du Bois-Reymond's Arch., 1887, Suppl.

[3] Maly's Jahresber., Bd. 19, S. 315.

[4] *Ibid.*, 20, S. 268

[5] Zeitschr. f. Biologie, Bd. 27.

The quantitative analyses of the human spleen by OIDTMANN[2] give the following results: In men he found 750–694 p. m. water and 250–306 p. m. solids. In that of a woman he found 774.8 p. m. water and 225.2 p. m. solids. The quantity of inorganic bodies was in men 4.9–7.4 p. m., and in women 9.5 p. m.

In regard to the pathological processes going on in the spleen we must specially recall the abundant re-formation of leucocytes in leucæmia and the appearance of amyloid substance (see page 57).

The physiological functions of the spleen are little known with the exception of its importance in the formation of leucocytes. Some consider the spleen as an organ for the dissolution of the red blood-corpuscles, and the occurrence of the above-mentioned deposit rich in iron seems to confirm this view. Other investigators regard the spleen as a blood-forming organ. Several investigators claim the occurrence of nucleated preliminary steps in the formation of red corpuscles in the spleen or of younger red corpuscles in the blood of the splenic vein.

The spleen has also been claimed to play an important part in digestion. The organ is known to enlarge after a meal, and this enlargement is thought by SCHIFF[3] and HERZEN[4] to be connected with the filling of the pancreas with enzymes. According to the above-mentioned investigators, after the extirpation of the spleen the pancreas does not produce any enzyme which digests proteids, but HEIDENHEIM[5] and EWALD[6] have not been able to confirm this fact. According to later investigations of HERZEN,[7] an enzyme which digests proteids is produced in the spleen during its enlargement.

An increase in the quantity of uric acid eliminated has been observed by many investigators (see Chapter XV) in lineal leucæmia, while the reverse has been observed under the influence of quinin in large doses, which produces an enlargement of the spleen. We have here a rather positive proof that there is a close relationship between the spleen and the formation of uric acid. This relationship has lately been studied by HORBACZEWSKI.[1] He has shown that when the spleen pulp and blood of calves is allowed to act on each other, under certain conditions and temperature, in the presence of air, large quantities of uric acid are formed. Under other conditions he obtained from the spleen pulp only xanthin bases with no or very little uric acid. HORBACZEWSKI has also shown that the uric acid originates from the nucleins of the spleen, which yield uric acid and xanthin bases according to the experimental conditions.

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[1] Cit. from Hoppe-Seyler's Physiol. Chem., S. 720.

[2] Cit. from v. Gorup-Besanez, Lehrbuch, 4. Aufl., S. 719.

[3] Arch. f. Heilkunde, Bd. 3, Schweiz. Zeitschr. f. wiss. Med., 1862.

[4] Pflüger's Arch , Bd. 30, S. 295 and 308.

[5] L. Hermann's Handb. d. Physiol., Bd. 5, S. 206.

[6] Verhandl. d. physiol. Ges. in Berlin, 1878.

[7] Maly's Jahresber., Bd. 18, S. 192.

The spleen has the same property as the liver of retaining foreign bodies, metals and metalloids.

The Thymus. Besides proteids and substances belonging to the connective group, we find small quantities of *fat*, *leucin*, *succinic acid*, *lactic acid*, and *glucose*. The large quantity of *xanthin bodies*, chiefly *adenin*, is remarkable—1.79 p. m. in the fresh gland, or 19.19 p. m. in the dried substance (KOSSEL and SCHINDLER[2]). LILIENFELD[3] has found *inosit* and *protagon* in the cells of the thymus. The quantitative composition of the lymphocytes of the thymus of a calf is, according to LILIENFELD'S[4] analysis, as follows. The results are given in 1000 parts of the dried substance.

Proteids	17.6
Leuconuclein	687.8
Histon	86.7
Lecithin	75.1
Fat	40.2
Cholesterin	44 0
Glycogen	8.0

The dried substance of the leucocytes amounted to an average of 114.9 p. m. Potassium and phosphoric acid are prominent mineral constituents. LILIENFELD found KH_2PO_4 amongst the bodies soluble in alcohol. OIDTMANN[5] found 807.06 p. m. water, 192.74 p. m. organic and 0.2 p. m. inorganic substances in the gland of a child two weeks old.

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[1] Monatshefte f. Chem., 1889, and Wien. Sitzungsber. 1891, Math. Naturw. Klasse, Abthl. 3.

[2] Zeitschr. f. physiol. Chem., Bd. 13.

[3] *Ibid.*, Bd. 18, S. 473.

[4] L. c.

[5] Cit. from v. Gorup-Besanez, Lehrbuch, 4. Aufl., S. 732.

The Thyroid Gland. The chemical constituents of this gland are little known. Bubnow[1] has obtained a protein substance called by him "*thyreoproteine*," by extracting the gland with common-salt solution or by very dilute caustic potash. This body has about the same amount of nitrogen, but smaller amounts of carbon and hydrogen than, the proteids in general. The fluid found in the vesicle sometimes contains a *mucin-like substance* which is precipitated by an excess of acetic acid. Gourlay[2] could not find any mucin but only a nucleoalbumin in the thyroid gland of oxen. Besides these, other substances have been found in the extract of the glands, such as *leucin*, *xanthin*, *hypoxanthin*, *lactic* and *succinic acids*. Oidtmann[3] found in the thyroid gland of an old woman 822.4 p. m. water, 176.7 p. m. organic and 0.9 p. m. inorganic substances. He found 772.1 p. m. water, 223.4 p. m. organic and 4.5 p. m. inorganic substances in an infant two weeks old.

In "STRUMA CYSTICA" Hoppe-Seyler found hardly any proteid in the smaller glandular vessels, but an excess of *mucin*, while in the larger he found a great deal of *proteid*, 70–80 p. m.[4] *Cholesterin* is regularly found in such cysts, sometimes in such large quantities that the entire contents form a thick mass of cholesterin plates. Crystals of *calcium oxalate* also occur frequently. The contents of the struma cysts are sometimes of a brown color due to decomposed coloring matter, *methæmoglobin* (and hæmatin?). Bile-coloring matters have also been found in such cysts. (In regard to the *paralbumins* and *colloids* which have been found in struma cysts and colloid degeneration, see Chapter XIII.)

Little is known in regard to the functions of the thyroid gland. From a chemical standpoint the view is worth suggesting that the so-called myxœdema, which is a slimy infiltration or abundant extuberance of the connective tissue of the subcutaneous cell-tissue especially of the head and throat (besides other disturbances) stands in connection with the failing of the activity of the thyroid gland. Horsley and Halliburton[5] found in monkeys, but not in pigs, that the amount of mucin in the tissue was increased after extirpating the thyroid gland.

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[1] Zeitschr. f. physiol. Chem., Bd. 8.

[2] Journal of Physiol., Vol. 16.

[3] Cit. from v. Gorup-Besanez, Lehrbuch, S. 732.

[4] Hoppe-Sevler, Physiol. Chem., S. 721.

[5] Brit. Med. Journ., 1885; also Maly's Jahresber., Bd. 18, S. 324.

We have no explanation as to the action of the gland in these cases. In consideration of the very favorable therapeutical results which have been obtained in many cases of myxœdema by the injection of a watery or glycerin extract of the gland or the administration of the gland of sheep, it seems probable that myxœdema is caused by an intoxication produced by metabolic products, which are otherwise destroyed or made harmless by the gland.

The Suprarenal Capsule.—Besides proteids, substances of the connective tissue, and salts, we find in the suprarenal capsule *inosit*, *palmitin*, *lecithin*, *neurin*, and *glycero-phosphoric acid*, which last gives the poisonous properties of the watery extract of the gland (MARINO-ZUCO and GUARNIERI[1]), and some *leucin*, which is probably a decomposition product. The statement that *benzoic acid*, *hippuric acid*, and *biliary acids* occur in this gland could not be confirmed by STADELMANN.[2] In the medulla there have been found one or more *chromogens* which are converted into a red pigment by the action of air, light, warmth, haloid or metallic salts (VULPIAN, KRUKENBERG[3]). Pyrocatechin also probably occurs therein. Because of the amount of chromogen contained in the suprarenal body, a connection is claimed between the abnormal deposition of pigment in the skin, which is characteristic of ADDISON's disease, and the diseased changes which often occur in the suprarenal body.

Nothing positive is known as to the functions of the suprarenal capsule. The extirpation of the suprarenal capsule of a dog is always a fatal operation (LANGLOIS). Death is hastened by the injection of blood from an animal killed by this operation, while the blood from a healthy animal has no action. Perhaps we have here also to deal with an intoxication produced by metabolic products, which are made harmless or destroyed by the suprarenal capsules under normal conditions. The investigations of ABELOUS and LANGLOIS and others seem to confirm this view

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[1] Maly's Jahresber., Bd. 18, S. 231.

[2] Zeitschr. f. physiol. Chem., Bd. 18.

[3] Virchow's Arch., Bd. 101.

CHAPTER VIII.

THE LIVER.

THE liver, which is the largest organ of the body, stands in close relationship to the blood-forming organs. The importance of this organ in the physiological composition of the blood is evident from the fact that the blood coming from the digestive tract, laden with absorbed bodies, must circulate through the liver before it is driven by the heart through the different organs and tissues. It has been proved, at least for the carbohydrates, that an assimilation of the absorbed nutritive bodies which are brought to the liver by the blood of the portal vein takes place in this organ. The occurrence of synthetical processes in the liver has been positively proved by special observations. It is possible that in the liver certain ammonia combinations are converted into urea or uric acid (in birds), while certain products of putrefaction in the intestine, such as phenol, may be converted by synthesis into ethereal sulphuric acids by the liver (PFLÜGER and KOCHS[1]). The liver has also the property of removing and retaining heterogeneous bodies from the blood, and this is not only true of metallic salts, which are often retained by this organ, but also, as SCHIFF, LAUTENBERGER, JACQUES, HÉGER, and ROGER[2] have shown, the alkaloids are retained and are probably partially decomposed in the liver. Toxins are also retained by the liver and hence this organ has a protective action against poisons.

Even though the liver is of assimilatory importance and purifies the blood coming from the digestive tract, it is at the same time a secretory organ which eliminates a specific secretion, the bile, in the production of which the red blood-corpuscles are destroyed, or at least one of their constituents, the hæmoglobin. It is generally admitted that the liver acts contrariwise during fœtal life, at that time forming the red blood-corpuscles.

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[1] Pflüger's Arch., Bd. 20 and Bd. 23, S. 169.

[2] Roger, Action du foie sur les poisons (Paris, 1887); Bouchard, Leçons sur les autointoxications dans les Maladies (Paris, 1887); and E. Kotliar in Arch. des sciences biologique de St. Petersbourg, Tome 2, No. 4, p. 587.

There is no doubt that the chemical operations going on in this organ are manifold and must be of the greatest importance for the organism; but unfortunately we know very little about the kind and extent of these processes. Among them are two principal ones which will be fully treated in this chapter, after we have first described the constituents and the chemical composition of the liver. One of these processes seems to be of an assimilatory nature and refer to the formation of glycogen, while the other refers to the production and secretion of the bile.

The reaction of the liver-cell is alkaline during life, but becomes acid after death. This change is probably due to the formation of lactic acid, causing a coagulation of the proteids of the protoplasm of the cell. A positive difference between the albuminous bodies of the dead and the living, non-coagulated protoplasm has not been observed.

The *proteids* of the liver were first carefully investigated by PLÓSZ.[1] He found in the watery extract of the liver an *albuminous substance* which coagulates at + 45° C., also a *globulin* which coagulates at + 75° C., a *nucleoalbumin* which coagulates at + 70° C., and lastly a proteid body which is nearly related to *coagulated albumins* and which is insoluble in dilute acids or alkalies at the ordinary temperature, but dissolves on the application of heat, being converted into an albuminate. HALLIBURTON[2] has found two globulins in the liver-cells, one of which coagulates at 68–70° C., and the other at 45–50° C. He also found, besides traces of albumin, a nucleoalbumin (nucleoproteid) which contained 1.45% phosphorus and a coagulation-point of 60° C. The liver-cells contain, besides these proteids, a large quantity of difficultly soluble protein bodies (see PLÓSZ). ST. ZALESKI[3] has found in the liver a *proteid containing iron*, in which the iron is more or less strongly combined. It is unknown what relation this bears to the above-mentioned proteids.

The *fat* of the liver occurs partly as very small globules and partly, especially in nursing children and sucking animals, as also after food rich in fat, as rather large fat-drops. This infiltration of fat, which may be made so abundant by proper food that it appears similar in the highest degree to a pathological fatty liver, begins in the periphery of the acini and extends towards the centre. If the amount of fat in the liver is increased by an infiltration, the water decreases correspondingly, while the quantity of the other solids remains little changed. In fatty degeneration this is different. In this process the fat is formed from the protoplasm of the cell, and the quantity of the other solids is therefore diminished while the amount of water is only slightly changed. To illustrate this, we give below the results from a normal liver, and also the results obtained by PERLS[1] in fatty degeneration and fatty infiltration. The results are in 1000 parts.

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[1] Pflüger's Arch., Bd. 7.

[2] Journal of Physiol., Vol. 13, Suppl. 1892.

[3] Zeitschr. f. physiol. Chem., Bd. 10, S. 486.

	Water.	Fat.	Remaining Solids.
Normal liver....................	770	20–35	207–195
Fatty degeneration.............	816	87	97
Fatty infiltration........... ...	616–621	195–240	184–145

Among the *extractive substances* besides *glycogen*, which will be treated of later, we find rather large quantities of *xanthin bases.* KOSSEL[2] found in 1000 parts of the dried substance 1.97 p. m. *guanin*, 1.34 p. m. *hypoxanthin*, and 1.21 p. m. *xanthin*. *Adenin* is also contained in the liver. In addition there have been found *urea* and *uric acid* (especially in birds), and indeed in larger quantities than in the blood, *paralactic acid*, *leucin*, *jecorin*, and *cystin.* In pathological cases *inosit* and *tyrosin* have been detected. The occurrence of *bile-coloring matters* in the liver-cell under normal conditions is doubtful; but in retention of the bile the cells may absorb the coloring matter and become colored thereby.

Jecorin was first found by DRECHSEL[3] in the liver of a horse, and later by BALDI[4] in the liver and spleen of other animals, in the muscles and blood of the horse, and in the human brain. It contains sulphur and phosphorus, but its constitution is not positively known. Jecorin dissolves in ether, but is precipitated from this solution by alcohol. It reduces copper oxide, and it solidifies after boiling with alkalies to a gelatinous mass. It may lead to errors in the investigations of organs or tissues, for it can easily be mistaken for lecithin on account of its solubilities and because it contains phosphorus.

The *mineral bodies* of the liver consist of phosphoric acid, potassium, sodium, alkaline earths, and chlorine. The potassium is in excess of the sodium. Iron is a regular constituent of the liver, but in very variable amounts, 0.3–11.8 p. m. calculated for the dried substance of the liver of different animals (ST. ZALESKI [1]). BUNGE [2] has found 0.01–0.355 p. m. iron in the blood-free liver of young cats and dogs. This was calculated on the liver substance freshly washed with a 1% NaCl solution. Calculated on 10 kilos bodily weight, the iron in the livers amounted to 3.4–80.1 mgm.

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[1] Centralbl. f. d. med. Wissensch., Bd. 11, S. 801.

[2] Zeitschr. f. physiol. Chem., Bd. 8, S. 408.

[3] Ber. d. sächs. Ges. d. Wissensch., 1886, S. 44.

[4] Du Bois-Reymond's Arch., Physiol. Abth., 1887. Suppl. S. 100.

The richness of the liver of new-born animals in iron is of special interest; a condition which follows from the analyses of ST. ZALESKI, but especially studied by KRÜGER, MEYER, and PERNOU.[3] In oxen and cows they found 0.246–0.276 p. m. iron (calculated on the dry substance), and in the cow fœtus about ten times as much. The liver-cells of a calf a week old contain about seven times as much iron as the full-grown animal; the quantity sinks in the first four weeks of life, when it about reaches the same amount as in the grown animal. LAPICQUE [4] has also found that in rabbits the quantity of iron in the liver steadily diminishes from the eighth day to three months after birth, namely, from 10 to 0.4 p. m., calculated on the dry substance. "The fœtal liver-cells bring an abundance of iron into the world to be used up, within a certain time, for a purpose not well known." A part of the iron exists as phosphate, and the greater part in combination with the protein bodies (ST. ZALESKI). F. KRÜGER [5] has determined the quantity of calcium in the liver-cells of oxen in various stages of development, and has found that the average quantity was only 0.71 p. m. of the dried substance in full-grown oxen and 1.23 p. m. in calves. In the fœtus of the cow it is lower than in calves, but it shows two maxima during fœtal life, one in the first to the fifth month, and the other in the tenth month, of pregnancy. At these times the liver-cells contain about 45% more calcium than in full-grown oxen. During pregnancy the iron and calcium are antagonistic; namely, an increase in the quantity of calcium causes a diminution in the iron, and an increase in the iron causes a decrease in the calcium. KRÜGER found 23.8 p. m. sulphur, 12.8 p. m. phosphorus, and 0.55 p. m. iron in the liver-cells of adult persons and 35.6 p. m.

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[1] L. c., S. 464–479.

[2] Zeitschr. f. physiol. Chem., Bd. 17, S. 78.

[3] Zeitschr. f. Biologie, Bd. 27, S. 439.

[4] Maly's Jahresber., Bd. 20, S. 268.

[5] Zeitschr. f. Biologie, Bd. 31.

sulphur, 15.4 p. m. phosphorus, and 3.14 p. m. iron in those of new-born infants. Copper seems to be a physiological constituent. Foreign metals, such as lead, zinc, and others (also iron), are easily taken up and retained for a long time by the liver.

v. BIBRA[1] found in the liver of a young man who had suddenly died, 762 p. m. water and 238 p. m. solids, consisting of 25 p. m. fat, 152 p. m. proteid and gelatin-forming substance, and 61 p. m. extractive substances.

Glycogen and its Formation.

Glycogen was discovered by BERNARD and HENSEN[2] independently of each other. It is a carbohydrate closely related to the starches or dextrins with the general formula $C_6H_{10}O_5$, perhaps $6(C_6H_{10}O_5) + H_2O$ (KÜLZ and BORNTRÄGER[3]). The largest quantities are found in the liver of full-grown animals, and smaller quantities in the muscles (BERNARD, NASSE[4]). It is found in very small quantities in nearly all tissues of the animal body. Its occurrence in lymphoid cells, blood, and pus has been mentioned in previous chapter, and it seems to be a regular constituent of all cells capable of development. Glycogen was first shown to exist in embryonic tissues by BERNARD and KÜHNE,[5] and it seems on the whole to be a constituent of such tissues in which a rapid cell-formation and cell-development is taking place. It is also present in rapidly forming pathological swellings (HOPPE-SEYLER[6]). Certain animals, as certain muscles, are very rich in glycogen (BIZIO[7]). Glycogen also occurs in the plant kingdom, especially in many fungi.

The quantity of glycogen in the liver, as also in the muscles, depends essentially upon the food. In starvation it disappears nearly completely after a short time, but more rapidly in small than in large animals. According to the old views it disappears earlier from the muscles than from the liver. According to the later determinations of ALDEHOFF[1] on hens, pigeons, rabbits, cats, and horses, which have been confirmed by KÜLZ and HERGENHAHN[2] and others, the muscle glycogen has a greater resistance to destruction than liver glycogen. After partaking of food especially abundant in carbohydrates, the liver becomes rich again in glycogen, the greatest increment occurring 14 to 16 hours after eating (KÜLZ[3]). HERGENHAHN found on experiments with hens that the appearance of the maximum of glycogen in the liver was also dependent upon the quantity of carbohydrates partaken of. The maximum of liver glycogen was reached on supplying 10 gms. cane-sugar in 12 hours, and after 30 gms. in 20 hours. The maximum of muscle glycogen is reached after 20–24 hours, independently of the quantity of cane-sugar supplied. The quantity of liver glycogen may amount to 120–160 p. m. after partaking of large quantities of carbohydrates. Ordinarily it is considerably less, namely, 12–30 to 40 p. m.

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[1] See v. Gorup-Besanez, Lehrbuch, 4. Aufl., S. 711.

[2] Cl. Bernard, Comp. rend., Tome 44, p. 578; and Hensen, Virchow's Arch., Bd. 11, S. 395.

[3] Pflüger's Arch., Bd. 24, S. 19.

[4] *Ibid.*, Bd. 2, S. 97.

[5] See Kühne, Lehrb. d. physiol. Chem., 1868, S. 307.

[6] Pflüger's Arch., Bd. 7, S. 409.

[7] Comp. rend., Tome 62, p. 675.

The quantity of glycogen of the liver (and also the muscles) is also dependent upon rest and activity, because during activity the quantity diminishes. KÜLZ[4] has shown that by hard work the quantity of glycogen in the liver (of dogs) is reduced to a minimum in a few hours. The muscle glycogen does not diminish to the same extent as the liver glycogen. KÜLZ was able to completely consume the liver as well as the muscle glycogen of a rabbit in 3–5 hours by qualified strychnin poisoning.

Glycogen forms an amorphous, white, tasteless, and inodorous powder. It gives an opalescent solution with water which, when allowed to evaporate in the water-bath, forms a pellicle over the surface that disappears again on cooling. The solution is dextrogyrate, $(\alpha)\,D = +196^{\circ}.63$ (HUPPERT[5]). The specific rotatory power is given somewhat differently by various investigators. A solution of glycogen, especially on the addition of NaCl, is colored wine-red by iodine. It may hold copper oxyhydrate in solution in alkaline liquids, but does not reduce it. A solution of glycogen in water is not precipitated by potassium-mercuric iodide and hydrochloric acid, but is precipitated by alcohol (on the addition of NaCl when necessary) or ammoniacal lead acetate. It gives a white granular precipitate of benzoyl glycogen with benzoyl chloride and caustic soda. Glycogen is not decomposed on prolonged boiling with dilute caustic potash, but it seems to be changed slightly (VINTSCHGAU and DIETL[1]). By diastatic enzymes glycogen is converted into maltose or dextrose, depending upon the nature of the enzyme. It is transformed into dextrose by dilute mineral acids.

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[1] Zeitschr. f. Biologie, Bd. 25, S. 137. Contains a summary of the literature.

[2] *Ibid.*, Bd. 27, S. 214.

[3] Pflüger's Arch., Bd. 24, S. 1–114. This important article contains numerous data in regard to the literature of the glycogen question.

[4] Pflüger's Arch., Bd. 24, and "Beiträge zur Kenntniss des Glykogens." C. Ludwig's Festschrift. Marburg, 1891.

[5] Zeitschr. f. physiol. Chem., Bd. 18, S. 137.

The preparation of pure glycogen (simplest from the liver) is generally performed by the method suggested by BRÜCKE, of which the main points are the following: Immediately after the death of the animal the liver is thrown into boiling water, then finely divided and boiled several times with fresh water. The filtered extract is now sufficiently concentrated, allowed to cool, and the proteids removed by alternately adding potassium-mercuric iodide and hydrochloric acid. The glycogen is precipitated from the filtered liquid by the addition of alcohol until the liquid contains 60 vols. per cent. The glycogen is first washed on the filter with 60% and then with 95% alcohol, then treated with ether and dried over sulphuric acid. It is always contaminated with mineral substances. To be able to extract the glycogen from the liver or especially from muscles and other tissues completely, which is essential in a quantitative estimation, these parts must first be boiled for a few hours with a dilute solution of caustic potash, say 4 gms. KOH to 100 gms. liver and 400 c.c. water (KÜLZ).

Proteid-free glycogen may be prepared according to the method suggested by HUIZINGA,[2] in which the liver tissue is extracted with a mixture of equal volumes of a saturated mercuric-chloride solution and ESBACH's reagent (10 gm. picric acid and 20 gms. citric acid in a liter). The glycogen is precipitated by alcohol and treated with alcohol and ether.

The quantitative estimation is best performed according to the described method of BRÜCKE-KÜLZ.[3] It is to be observed that it is necessary to heat the liver for 2–3 hours and muscle 4–8 hours with caustic-potash solution. This liquid must not be concentrated too far, and must not contain more than 2% caustic potash. It is neutralized by hydrochloric acid and precipitated by the alternate addition of potassium-mercuric iodide and hydrochloric acid. The precipitate must be removed from the filter at least four times, suspended in water with the addition of a few drops HCl and potassium-mercuric iodide, and refiltered so that all the glycogen is obtained in the filtrates. These are then precipitated with double their volume of alcohol, filtered after 12 hours, the precipitate dissolved in a little warm water, treated on cooling with HCl and potassium-mercuric iodide, filtered, and the filtrate again precipitated with alcohol. Filter and carefully wash the contents of the filter with alcohol and ether, dry, weigh, and incinerate to determine the quantity of ash present.

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[1] Pflüger's Arch., Bd. 13, S. 253.

[2] Pflüger's Arch., Bd. 61.

[3] See R. Külz, Zeitschr. f. Biologie, Bd. 22, S. 161.

It sometimes happens that the liquid, after complete precipitation of the proteids with HCl and potassium-mercuric iodide, is cloudy and does not filter clear. In this case add 2–2½ vols. 95% alcohol according to PFLÜGER's[1] suggestion. After the liquid becomes clear and the precipitate has settled it can be filtered. The precipitate is dissolved in a 2% caustic-potash solution and again precipitated by hydrochloric acid and potassium-mercuric iodide. Then proceed as above described.

The new method as suggested by FRÄNKEL,[2] in which the glycogen is extracted from the tissues by a 3–4% water solution of trichloracetic acid, seems not to be reliable, according to WEIDENBAUM.[3]

Numerous investigators have endeavored to determine the origin of glycogen in the animal body. It is positively established by the unanimous observations of many investigators[4] that the varieties of *sugars* and their anhydrides, *dextrins* and *starches*, have the property of increasing the quantity of glycogen in the body. The statements are somewhat disputed in regard to the action of the pentoses. CREMER[5] found that various pentoses such as rhaminose, xylose, and arabinose have a positive influence on the glycogen formation in rabbits and hens, and SALKOWSKI[6] obtained the same result on feeding rabbits and a hen on arabinose. FRENTZEL[7] found, on the contrary, no glycogen formation on feeding xylose to a rabbit which had previously been made glycogen-free by strychnin poisoning.

The hexoses, and the carbohydrates derived therefrom, do not all possess the ability of forming or accumulating glycogen to the same extent. Thus C. VOIT[8] and his pupils have shown that dextrose has a more powerful action than cane-sugar, while milk-sugar acts disproportionately less (in rabbits and hens) than dextrose, lævulose, cane-sugar, and maltose. The following substances when introduced into the body also increase the quantity of glycogen in the liver: *glycerin*, *gelatin*, *arbutin*, and also, according to the investigations of KÜLZ,[1] *erythrit*, *quercit*, *dulcit*, *mannit*, *inosit*, *allyl* and *crotyl alcohols*, *glycuronic anhydride*, *saccharic acid*, *mucic acid*, *sodium tartrate*, *saccharin*, *isosaccharin*, and *urea*. *Ammonium carbonate*, *glycocoll*, and *asparagin* may also, according to RÖHMANN,[2] cause an increase in the amount of glycogen in the liver. According to NEBELTHAU[3] other ammonium salts and certain amides, also certain *narcotics*, *hypnotics*, and *antipyretics*, produce an increase in the glycogen of the liver. This action of the antipyretics (especially antipyrin) had been shown by LÉPINE and PORTERET.[4]

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[1] Pflüger's Arch., Bdd. 53 and 55.

[2] *Ibid.*, Bdd. 52 and 55.

[3] *Ibid.*, Bdd. 54 and 55.

[4] In reference to the literature on this subject see E. Külz, Pflüger's Arch., Bd. 24, and Ludwig-Festschrift, 1891; Wolffberg, Zeitschr. f. Biologie, Bd. 12, and C. Voit, *ibid.*, Bd. 28, S. 245.

[5] Zeitschr. f. Biologie, Bd. 29.

[6] Centralbl. f. d. med. Wissensch., 1893, No. 11.

[7] Pflüger's Arch., Bd. 56.

[8] Zeitschr. f. Biologie, Bd. 28.

The fats, notwithstanding the above-mentioned action of glycerin, have no action on the quantity of glycogen in the liver, according to the statements of most investigators. The views in regard to the action of proteids have been very contradictory in the past. It is undoubtedly settled from many observations that the proteids also increase the liver glycogen. Amongst these observations we must include certain feeding experiments with boiled beef (NAUNYN) or blood fibrin (v. MERING), and especially the very careful experiments made by E. KÜLZ[5] on hens with pure proteids such as casein, seralbumin, and ovalbumin. WOLFFBERG[6] has also shown that a more abundant accumulation of glycogen takes place after feeding with proteids and carbohydrates in proper proportions than with carbohydrate food with only a little proteid.

MIURA[7] has made experiments to demonstrate the rôle of the inulin as a glycogen-former in starving rabbits. In certain cases the quantity of glycogen was increased, in others, on the contrary, not affected. The inconstancy of the results of these tests may be dependent upon the fact that the inulin introduced was only partly or only slowly transformed into lævulose, and hence the absorbed sugar could not always cause an accumulation of the glycogen. Miura also mentions the results of the older experiments and also gives the older literature.

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[1] E. Külz, Ludwig's Festschrift, 1891.

[2] Pflüger's Arch., Bd. 39.

[3] Zeitschr. f. Biologie, Bd. 28, S. 138.

[4] Comp. rend., Tome 106, p. 1023.

[5] Cit. Ludwig's Festschrift. The complete literature in regard to the glycogen formation from proteids will be found here.

[6] Zeitschr. f. Biologie., Bd. 16, S. 266.

[7] *Ibid.*, Bd. 32.

If we raise the question as to the action of the various bodies in the accumulation of glycogen in the liver we must call to mind that a reformation of glycogen takes place in this organ, and also a consumption of the same.[1] An accumulation of glycogen may be caused by an increased formation of glycogen, but also by a diminished consumption, or by both.

We do not known how all the above-mentioned various bodies act in this regard. Certain of them probably have a retarding action on the transformation of glycogen in the liver, while others perhaps are more combustible and in this way protect the glycogen. Some probably excite the liver-cells to a more active glycogen formation, while others yield material from which the glycogen is formed and are *glycogen-formers* in the true sense of the word. The knowledge of these last-mentioned bodies is of the greatest importance in the question as to the origin of glycogen in the animal body, and the chief interest attaches itself to the question, to what extent are the two chief groups of food, the proteids and carbohydrates, glycogen-formers?

The great importance of the carbohydrates in the formation of glycogen has given rise to the opinion that the glycogen in the liver is produced from other carbohydrates (glucose) by a synthesis in which water separates with the formation of an anhydride (Luchsinger and others). This theory (*anhydride theory*) has found opponents because it neither explains the formation of glycogen from such bodies as proteids, carbohydrates, gelatin, and others, nor the circumstance that the glycogen is always the same independent of the properties of the carbohydrate introduced, whether it is dextro- or lævo-gyrate. It is therefore the opinion of many investigators that all glycogen is formed from proteid, and that this splits into two parts, one containing nitrogen and the other free from nitrogen: the latter is the glycogen. According to these views, the carbohydrates act only in that they spare the proteid and the glycogen produced therefrom (*sparing theory* of Weiss, Wolffberg, and others[2]).

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[1] See Wolffberg, l. c.

[2] See Wolffberg, l. c., in regard to these two theories.

In opposition to this view E. Voit,[1] by feeding experiments with rice, which is poor in nitrogen, and C. Voit[2] and his pupils, by tests with dextrose, lævulose, maltose, and cane-sugar, have shown that the quantity of glycogen stored up in the body, after partaking of large amounts of carbohydrates, is sometimes so large that it cannot be covered by the proteids decomposed during the same time. In these cases we must admit of glycogen formation from sugars. The investigations of C. Voit show that dextrose directly or lævulose either directly or after previous conversion into dextrose passes into glycogen in the liver. Maltose and cane-sugar must first probably be transformed into dextrose or invert-sugar in the intestinal tract. Milk-sugar and galactose seem, according to Kausch and Socin,[3] although contrary to the observations of Voit, to form glycogen directly if the absorption in the intestine is sufficiently abundant.

There is no doubt that feeding with pure proteids leads to an accumulation of glycogen, and at the present time we must admit that glycogen can be formed from proteids as well as from carbohydrates.

The manner in which glycogen is formed from proteids is not known. The view held by certain investigators that carbohydrates split off directly from the genuine proteids has not sufficient basis, and therefore the glycogen formation is often explained according to Pflüger,[4] by a synthesis from the proteids after a complex cleavage.

Like the carbohydrates in general, so has glycogen without any doubt a great importance in the formation of heat and development of energy in the animal body. The possibility of the formation of fat from glycogen must not be denied. Glycogen is generally considered as accumulated reserve food in the liver and formed in the liver-cells. Where does the glycogen existing in the other organs, such as the muscles, originate? Is the glycogen of the muscles formed on the spot or is it transmitted to the muscles by the blood? These questions cannot yet be answered with positiveness, and the investigations on this subject by different experimenters[5] have given contradictory results. The later experiments of Külz,[6] in which he studied the glycogen formation by passing blood containing cane-sugar through the muscle, has led to no conclusive results.

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[1] Zeitschr. f. Biologie, Bd. 25, S. 543.

[2] *Ibid.*, Bd. 28.

[3] Arch. f. exp. Path. u. Pharm., Bd. 31.

[4] Pflüger's Arch., Bd. 42.

[5] See Minkowski and Laves, Arch. f. exp. Path. u. Pharm., Bd. 23.

[6] Zeitschr. f. Biologie, Bd. 27.

If we consider that the blood and lymph contain a diastatic enzyme which transforms glycogen into dextrose, and also that the glycogen regularly occurs in the form-elements and is not dissolved in the fluids, it seems probable that the glycogen is not transmitted by the blood to the organs in solution, but perhaps more likely, if the leucocytes do not act as carriers, is formed on the spot from the dextrose. The glycogen formation seems to be a general function of the cells. In adults the liver, which is very rich in cells, has the property, on account of its anatomical position, of transforming large quantities of dextrose into glycogen.

The question now arises whether there is any foundation for the statement that the liver glycogen is transformed into dextrose.

As first shown by BERNARD and repeated by many investigators, the glycogen in a dead liver is gradually changed into dextrose, and this sugar formation is caused, as BERNARD supposed and ARTHUS and HUBER[1] proved, by a diastatic enzyme. This post-mortem sugar formation led BERNARD to the assumption of the formation of sugar from glycogen in the liver during life. BERNARD[2] suggested the following arguments for this theory: The liver always contains some sugar under physiological conditions, and the blood from the hepatic vein is always somewhat richer in sugar than the blood from the portal vein. The correctness of either or both of these statements has been disputed by many investigators. PAVY, RITTER, SCHIFF, EULENBERG, LUSSANA, ABELES, and others deny the occurrence of dextrose in the liver during life, and also the greater amount of dextrose in the blood from the hepatic vein is disclaimed by them and certain other investigators. A few investigators claim that a greater amount of sugar may occur in the hepatic vein under certain circumstances, and they consider in these cases that it is caused by the operation.

The doctrine as to the physiological formation of sugar in the liver has obtained an energetic advocate in SEEGEN.[3] He maintains, after numerous experiments, that the liver regularly contains considerable amounts of sugar. He has observed an increase of 3% in the quantity of dextrose in the liver of a dog kept alive by passing arterial blood through the organ, and lastly he has also found in a very great number of experiments on dogs that the blood from the hepatic vein always contains more—even double as much—sugar than the blood from the portal vein.

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[1] Arch. de physiol., (5) Tome 4.

[2] In regard to the literature on sugar formation in the liver see Bernard, Leçons sur le diabète. Paris, 1877. Seegen, Die Zuckerbildung im Thierkörper. Berlin, 1890. M. Bial, Pflüger's Arch., Bd. 55, S. 434.

[3] See Seegen, Die Zuckerbildung im Thierkörper. Berlin, 1890.

Although SEEGEN energetically espouses the doctrine of BERNARD as to the vital sugar formation in the liver, still it deviates essentially from BERNARD in that he claims the dextrose is not derived from the glycogen. According to SEEGEN the sugar is formed from peptones and fat. The observations on which he bases this view seem hardly to be correct,[1] according to the control experiments made by many investigators. The statement of LÉPINE[2] as to the occurrence of an enzyme in the blood which has the property of transforming peptone into sugar could not be substantiated by BIAL.

The circumstance that the blood-sugar rapidly sinks to ½–⅓ of its original quantity, or even disappears when the liver is cut out of the circulation, speaks for a vital formation of sugar in the liver (SEEGEN, BOCK, and HOFFMANN[3]). In geese whose livers were removed from the circulation MINKOWSKI[4] found no sugar in the blood after a few hours. We will also learn shortly of certain poisons and operative changes which may cause an abundant elimination of sugar, but only when the liver contains glycogen. If we recall the fact shown by RÖHMANN and BIAL[5] that the lymph as well as the blood contains a diastatic enzyme, then several reasons speak for the view of BERNARD that the post-mortem formation of sugar from the glycogen in the liver is a continuation of the vital process. Although it is unanimous that the post-mortem sugar formation is produced by a diastatic enzyme, still several investigators, such as DASTRE and NOEL-PATON,[6] are of the view that sugar formation is not caused in life by an enzyme, but by a vital process of the cell protoplasm.

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[1] A compilation of these control experiments may be found in Bial, Pflüger's Arch., Bd. 55.

[2] Compt. rend., Tome 115 and 116.

[3] See Seegen, l. c., pp. 182–184.

[4] Arch. f. exp. Path. u. Pharm., Bd. 21.

[5] See pp. 124 and 181, this book.

[6] See Noel-Paton, On Hepatic Glycogenesis. Phil. Trans. of the Roy. Soc. London, vol. 185, B. 1894.

The relationship of the sugar eliminated in the urine under certain conditions, such as in diabetes mellitus, certain intoxications, lesions of the nervous system, etc., to the glycogen of the liver is also an important question.

It does not enter into the plan and scope of this book to enter into detail into the various views in regard to glycosuria and diabetes. The appearance of dextrose in the urine is a symptom which may have essentially different causes, depending upon different circumstances. Only a few of the most important points will be mentioned.

The blood contains always about an average of 1.5 p. m., while the urine at most contains only traces. When the quantity of sugar in the blood rises to 3 p. m. or above, then sugar passes into the urine. The kidneys have the property to a certain extent of preventing the passage of blood-sugar into the urine; and it follows from this that an elimination of sugar in the urine may be caused partly by a reduction or suppression of this above-mentioned activity and partly also by an abnormal increase of the quantity of sugar in the blood.

The first seems, according to v. MERING and MINKOWSKI, to be the case in phlorhizin[1] diabetes. v. MERING has found that a strong glycosuria appears in man and animals on the administration of the glucoside phlorhizin, and that the quantity of sugar in the blood is not increased, but somewhat diminished. In this form of diabetes we have, according to MINKOWSKI, abnormal processes in the kidneys. According to LEVENE[2] phlorhizin diabetes is not produced by an increased elimination of sugar by the kidneys, but more likely an increased formation of sugar in these organs. He found generally more sugar in the venous blood of the kidneys than in the arterial blood, and he also found considerably more sugar after injection of phlorhizin than under normal conditions. He agrees with the observations of other investigators such as PRAUSNITZ, CREMER, and RITTER, that in phlorhizin diabetes the sugar is formed from the protein substances. All other forms of glycusoria or diabetes depend, on the contrary, as far as known, to an increased quantity of sugar in the blood, namely, a *hyperglucæmia.*

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[1] In regard to the literature on phlorhizin diabetes see: v. Mering, Zeitschr. f. klin. Med., Bdd. 14 and 16; Minkowski, Berl. klin. Wochenschr., 1892, No. 5, and Arch. f. exp. Path. u. Pharm., Bd. 31; Moritz and Prausnitz, Zeitschr. f. Biologie., Bdd. 27 and 29; Külz and Wright, *ibid.*, Bd. 27, S. 181; Cremer and Ritter, *ibid.*, Bdd. 28 and 29.

[2] Journal of Physiol., Vol. 17.

A hyperglucæmia may be caused in various ways. It may be caused, for example, by the introduction of more sugar than the body can destroy.

The property of the animal body to assimilate the different varieties of sugar has naturally a limit. If too much sugar is introduced into the intestinal tract at one time, so that the so-called assimilation limit (see Chapter XIX on absorption) is overreached, then the excess of absorbed sugar passes into the urine. This form of glycosuria is called *alimentary glycosuria,*[1] and it is caused by the passage of more sugar into the blood than the liver and other organs can destroy.

As the liver cannot transform all the sugar into glycogen which comes to it in alimentary glycosuria, it is possible that a glycosuria may be brought about by the activity of the liver to transform sugar into glycogen being changed or reduced by disease. It is difficult to state in how far such a glycosuria occurs, but according to SEEGEN the lighter forms of diabetes are produced in this way.

We differentiate between light and severe forms of diabetes. In the first the urine only contains sugar when carbohydrates are taken as food, while in the other case the urine contains sugar even with food entirely free from carbohydrates. According to the view of SEEGEN,[2] in light forms of diabetes the liver is incapable of transforming all the carbohydrates introduced into glycogen, or to utilize this in a proper way, and the activity of the liver-cells is also reduced or changed in these cases. This view is nevertheless hardly based on sufficient proof.

A hyperglucæmia, which passes into a glycosuria, may also be brought about by an excessive formation of sugar from the glycogen within the animal body.

The so-called *piqure*, and also probably those glycosurias which occur after other lesions of the nervous system, belong to the above group of glycosurias. The glycosuria produced on poisoning with carbon monoxide, curare, strychnin, morphin, etc., also belong to this group. That the glycosuria produced in these cases is due to an increased transformation of the glycogen follows from the fact that no glycosuria appears, under the above-mentioned circumstances, when the liver has been previously made free from glycogen by starvation or other means.[1]

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[1] See Moritz, Arch. f. klin. Med., Bd. 46, 1890.

[2] Die Zuckerbildung, etc. Lecture 15.

A hyperglucæmia with glycosuria may also be caused by a decreased activity of the animal body to consume or destroy the sugar. In this case the sugar must accumulate in the blood, and the formation of diabetes mellitus is now generally explained by this process.

The inability of diabetics to destroy or consume the sugar does not seem to be connected with any decrease in the oxidation energy of the cells, as both varieties of sugar, dextrose and lævulose, both of which can be oxidized with the same readiness, act differently in the body of diabetics. Lævulose is, according to KÜLZ[2] and other investigators, contrary to dextrose, utilized to a great extent in the organism, and may even cause a deposit of glycogen in the liver in animals with pancreas-diabetes (MINKOWSKI[3]). In this diabetes the ability of the cells to utilize the dextrose is diminished, and this diminution of ability seems to be in some way dependent upon the pancreas. The investigations of MINKOWSKI, v. MERING, DOMENICIS, and later by other investigators[4] have shown that a true diabetes of a severe kind is caused by the total extirpation of the pancreas of many animals, especially dogs. As in man in severe forms of diabetes, so also in dogs with pancreas-diabetes an abundant elimination of sugar takes place even on the complete exclusion of carbohydrates in the food, and the formation of sugar in these cases is derived from the protein substances. It seems in man with diabetes that the ability of the sugar destruction is never quite arrested; in dogs with pancreas-diabetes MINKOWSKI and v. MERING, as also HEDON,[5] have been able, in a few cases, to detect that the total quantity of sugar introduced with the food passed into the urine.

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[1] See Bock, Pflüger's Arch., Bd. 5; Bock and Hoffmann, Expt. Studien über Diabetes (Berlin, 1874). Cl. Bernard, Leçons sur le diabète (Paris); T. Araki, Zeitschr. f. physiol. Chem., Bd. 15, S. 351.

[2] Beiträge zur Path. und Ther. des Diabetes mellitus Marburg, 1874.

[3] Arch. f. exp. Path. u. Pharm., Bd. 31.

[4] See Minkowski, Untesuchrungen über Diabetes mellitus nach Exstirpation des Pankreas (Leipzig, 1893), and the chapter on diabetes in v. Noorden's Lehrbuch der Path. des Stoffwechsels (Berlin, 1893), which contains a very copious index of the literature. In regard to diabetes see also Cl. Bernard, Leçons sur le diabète (Paris), and Seegen, Die Zuckerbildung im Thierkörper (Berlin, 1890).

[5] Arch. de Physiol., (5) Tome 5.

Artificial pancreas-diabetes may also in other respects present exactly the same picture as diabetes in man; and as in the past we have always looked to the liver for the cause of diabetes, our attention is now more and more called to the pancreas. We do not know in what respect the pancreas stands to diabetes. We will refer to this question again in a subsequent chapter (Chapter IX).

The Bile and its Formation.

By the establishment of a biliary fistula, an operation which was first performed by SCHWANN[1] in 1844 and which has been improved lately by DASTRE,[2] it is possible to study the secretion of the bile. This secretion is continuous, but with varying intensity. It takes place under a very low pressure; therefore an apparently unimportant hindrance in the outflow of the bile, namely, a stoppage of mucus in the exit or the secretion of large quantities of viscous bile, may cause stagnation and absorption of the bile by means of the lymphatic vessels (absorption icterus).

The quantity of bile secreted in the 24 hours in dogs can be exactly determined. The quantity secreted by different animals varies, and the limits are 2.9–36.4 gm. bile per kilo of weight in the 24 hours.[3]

The statements as to the extent of bile secretion in man are few and not to be depended on. RANKE[4] found (using a method which is not free from criticism) a secretion of 14 gm. bile with 0.44 gm. solids per kilo in 24 hours. NOEL-PATON[5] observed a 51-year-old woman with biliary fistula for 23 days, and found an average of 638 cc. with 8.378 gm. solids in 24 hours. MAYO-ROBSON,[6] whose observations on a woman 42 years old with biliary fistula extended over 15 months, found an average of 862 cc. for the 24 hours' bile secretion. The AUTHOR[7] found 650 cc. as the maximum quantity in a man and 950 cc. in a woman. Such determinations are of doubtful value, because in most cases it follows from the composition of the collected bile that we are not dealing with a secretion of normal liver-bile.

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[1] Arch. f. Anat. und Physiol., 1844.

[2] Arch. de Physiol., Tome 22.

[3] In regard to the quantity of bile secreted in animals see Heidenhain, Die Gallenabsonderung in Hermann's Handbuch der Physiol., Bd. 5, and Stadelmann, Der Icterus und seine verschiedenen Formen (Stuttgart, 1891).

[4] Die Blutvertheilung und der Thätigkeitswechsel der Organe. Leipzig, 1871.

[5] Rep. Lab. Roy. Coll. Phys. Edinb., Vol. 3.

[6] Proc. Roy. Soc., Vol. 47

[7] Nova Acta Reg. Soc. Scient. Upsala, Ser. 3, Vol. 16, 1893.

The quantity of bile secreted is, however, as specially shown by Stadelmann,[1] subject to such great variation even under physiological conditions that the study of these circumstances which influence the secretion is very difficult and uncertain. The contradictory statements by different investigators may probably be explained by this fact.

In starvation the secretion diminishes. According to Lukjanow[2] and Albertoni,[3] under these conditions the absolute quantity of solids decreases, while the relative quantity increases. After partaking of food the secretion increases again. The statements are contradictory in regard to the time necessary after partaking of food before the secretion reaches its maximum. After a careful examination and compilation of all the existing statements Heidenhain[4] has come to the conclusion that in dogs the curve of rapidity of secretion shows two maxima, the first at the 3d to 5th hour, and the second at the 13th to 15th hour, after partaking of food.

According to the older statements, the proteids, of all the different foods, cause the greatest secretion of bile, while the carbohydrates diminish, or at least excite much less than the proteids.

It is nevertheless positive that an increase in the bile secretion takes place after a continuous over-abundant meat diet. We are by no means agreed as to the action of the fats. While many older investigators have not observed any increase, but rather the reverse, in the secretion of bile after feeding with fats, the newer experiments made by Rosenberg[5] show that the fats have a more powerful exciting action on the secretion of bile than the other foods, and that olive-oil is a strong cholagogue. This statement seems, according to the investigations of Mandelstamm,[6] not to be sufficiently proven.

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[1] Der Icterus.

[2] Zeitschr. f. physiol. Chem., Bd. 16.

[3] Recherches sur la sécrétion biliaire. Turin, 1893.

[4] Hermann's Handb., Bd. 5, and Stadelmann, Der Icterus.

[5] Pflüger's Arch., Bd. 46.

[6] Ueber den Einfluss einiger Arzneimittel auf Sekretion und Zusammensetzung der Galle. Dissert. Dorpat, 1890. In regard to the action of various foods

The question whether there exist special medicinal bodies, so-called cholagogues, which have a specific exciting action on the secretion of bile has been answered in very different ways. Many, especially the older investigators, have observed an increase in the bile secretion after the use of certain therapeutic agents such as calomel, rhubarb, jalap, turpentine, olive-oil, sodium salicylate, etc., while others, especially the later investigators, have arrived at quite opposite results. From all appearances this contradiction is due to the great irregularity of the normal secretion, which may be readily mistaken in tests with therapeutic agents.

SCHIFF'S[1] view, that the bile absorbed from the intestinal canal increases the secretion of bile and hence acts as a cholagogue, seems to be a positively proven fact by the investigations of several experimenters.[2]

The bile is a mixture of the secretion of the liver-cells and the so-called mucus which is secreted by the glands of the biliary passages and by the mucous membrane of the gall-bladder. The secretion of the liver, which is generally poorer in solids than the bile from the gall-bladder, is thin and clear, while the bile collected in the gall-bladder is more ropy and viscous on account of the absorption of water and the admixture of "mucus," and cloudy because of the admixture of cells, pigments, and the like. The specific gravity of the bile from the gall-bladder varies considerably, being in man between 1.010 and 1.040. Its reaction is alkaline. The color changes in different animals: golden yellow, yellowish brown, olive-brown, brownish green, grass-green, or bluish green. Bile obtained from an executed person immediately after death is golden yellow or yellow with a shade of brown. Still cases occur in which fresh human bile has a green color. The ordinary post-mortem bile has a variable color. The bile of certain animals has a peculiar odor; as example, ox-bile has an odor of musk, especially on warming. The taste of bile is also different in different animals. Human as well as ox bile has a bitter taste with a sweetish after-taste. The

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on the secretion of bile see Heidenhain in Hermann's Handbuch, etc., and Stadelmann, Der Icterus.

[1] Pflüger's Arch., Bd. 3.

[2] See Stadelmann, Der Icterus, and the dissertations of his pupils, namely, Winteler, Expt. Beiträge zur Frage des Kreislaufes der Galle (Inaug. Diss. Dorpat, 1892), and Gertner, Expt. Beiträge zur Physiol. und Path. der Gallensekretion" (Inaug. Diss. Jurjew, 1893).

bile of the pig and rabbit has an intense persistent bitter taste. On heating bile to boiling it does not coagulate. It contains (in the ox) only traces of true mucin, and its ropy properties depend, it seems, chiefly on the presence of a nucleoalbumin similar to mucin (PAIJKULL[1]). The AUTHOR[2] has, on the contrary, found true mucin in human bile. The specific constituents of the bile are *bile-acids* combined with alkalies, *bile-pigments*, and besides small quantities of *lecithin*, *cholesterin*, *soaps*, *neutral fats*, *urea*, and *mineral substances*, chiefly sodium chloride, calcium, and magnesium phosphate, and iron. Traces of copper also occur.

Bile Salts. The thus-far best studied bile-acids may be divided into two groups, the *glycocholic* and *taurocholic* acid groups. As found by the AUTHOR,[3] a third group of bile-acids occur in the shark and probably also in other animals. They are rich in sulphur, and like the ethereal sulphuric acids they split off sulphuric acid on boiling with hydrochloric acid. All glycocholic acids contain nitrogen, but are free from sulphur and can be split with the addition of water into glycocoll (amido-acetic acid) and a nitrogen-free acid, cholalic acid. All taurocholic acids contain nitrogen and sulphur and are split, with the addition of water, into taurin (amido-ethylsulphonic acid) and cholalic acid. The reason of the existence of different glycocholic and taurocholic acids depends on the fact that there are several cholalic acids.

The different bile-acids occur in the bile as alkali salts, generally in combination with sodium, but in sea-fishes as potassium salts. In the bile of certain animals we find almost solely glycocholic acid, in others only taurocholic acid, and in other animals a mixture of both (see below).

All alkali salts of the biliary acids are soluble in water and alcohol, but insoluble in ether. Their solution in alcohol is therefore precipitated by ether, and this precipitate, with the proper care in manipulation, gives, for nearly all kinds of bile thus far investigated, rosettes or balls of fine needles or 4–6-sided prisms (PLATTNER's crystallized bile). Fresh human bile also crystallizes readily. The bile-acids and their salts are optically active and dextrorotatory. The former are dissolved by concentrated sulphuric acid at the ordinary temperature, forming a reddish-yellow liquid which has a beautiful green fluorescence. On carefully warming with concentrated sulphuric acid and a little cane-sugar, the bile-acids give a beautiful cherry-red or reddish-violet liquid. Pettenkofer's reaction for bile-acids is based on this behavior.

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[1] Zeitschr. f. physiol. Chem., Bd. 12.

[2] Nova Acta reg. soc. scient. Upsala, Ser. 3, Vol. 16.

[3] Investigation not published.

Pettenkoffer's *test for bile-acids* is performed as follows: A small quantity of bile in substance is dissolved in a small porcelain dish in concentrated sulphuric acid and warmed, or some of the liquid containing the bile-acids is mixed with concentrated sulphuric acid, taking special care in both cases that the temperature does not rise higher than 60–70° C. Then a 10% solution of cane-sugar is added, drop by drop, continually stirring with a glass rod. The presence of bile is indicated by the production of a beautiful red liquid, whose color does not disappear at the ordinary temperature, but becomes more bluish violet in the course of a day. This red liquid shows a spectrum with two absorption-bands, the one at *F* and the other between *D* and *E*, near *E*.

This extremely delicate test fails, however, when the solution is heated too high or if an improper quantity—generally too much—of the sugar is added. In the last-mentioned case the sugar easily carbonizes and the test becomes brown or dark brown. The reaction readily fails if the sulphuric acid contains sulphurous acid or the lower oxides of nitrogen. Many other substances, such as proteids, oleic acid, amyl alcohol, morphin, and others, give a similar reaction, and therefore in doubtful cases the spectroscopic examination of the red solution must not be forgotten.

Pettenkofer's test for the bile-acids depends essentially on the fact that furfurol is formed from the sugar by the sulphuric acid, and this body can therefore be substituted for the sugar in this test (Mylius). According to Mylius[1] and v. Udranszky[2] a 1 p. m. solution of furfurol should be used. Dissolve the bile, which must first be purified by animal charcoal, in alcohol. To each c. c. of alcoholic solution of bile in a test-tube add 1 drop of the furfurol solution and 1 c. c. conc. sulphuric acid, and cool when necessary so that the test does not become too warm. This reaction, when performed as described, will detect $\frac{1}{20}$–$\frac{1}{30}$ milligram cholalic acid (v. Udranszky). Other modifications of Pettenkofer's test have been proposed.

Glycocholic Acid. The constitution of that glycocholic acid, occurring in human and ox bile, which has been most studied is represented by the formula $C_{26}H_{43}NO_6$. Glycocholic acid is absent or nearly so in the bile of carnivora. On boiling with acids or alkalies this acid, which is analogous to hippuric acid, is converted into cholalic acid and glycocoll.

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[1] Zeitschr. f. physiol. Chem., Bd. 11, S. 492.

[2] *Ibid.*, Bd. 12, S. 370.

Glycocholic acid crystallizes in fine, colorless needles or prisms. It is soluble with difficulty in water (in about 300 parts cold and 120 parts boiling water), and is easily precipitated from its alkali-salt solution by the addition of dilute mineral acids. It is readily soluble in strong alcohol, but with great difficulty in ether. The solutions have a bitter but at the same time sweetish taste. The salts of the alkalies and alkaline earths are soluble in alcohol and water. The salts of the heavy metals are mostly insoluble or soluble with difficulty in water. The solution of the alkali salts in water is precipitated by sugar of lead, copper-oxide and ferric salts, and silver nitrate.

The preparation of pure glycocholic acid may be performed in several ways. We may precipitate the bile, which has been freed from mucus by means of alcohol and the alcohol removed by evaporation, by a solution of lead acetate. The precipitate is then decomposed by a soda solution and heat, evaporated to dryness, and the residue extracted with alcohol, which dissolves the alkali glycocholate. The alcohol is distilled from the filtered solution and the residue dissolved in water; this solution is now decolorized by animal charcoal, and the glycocholic acid precipitated from the solution by the addition of a dilute mineral acid. The acid may be obtained in crystals either from boiling water, on cooling, or from strong alcohol by the addition of ether. The reader is referred to more exhaustive works for other methods of preparation.

Hyo-glycocholic Acid, $C_{27}H_{43}NO_5$, is the crystalline glycocholic acid obtained from the bile of the pig. It is very insoluble in water. The alkali salts, whose solutions have an intense bitter taste without any sweetish after-taste, are precipitated by $CaCl_2$, $BaCl_2$, and $MgCl_2$, and may be salted out like a soap by Na_2SO_4 when added in sufficient quantity. Besides this acid there occurs in the bile of the pig still another glycocholic acid (JOLIN[1]).

The glycocholate in the bile of the rodent is also precipitated by the above-mentioned salts, but cannot, like the corresponding salt in the human or ox bile, be precipitated on saturating with a neutral salt (Na_2SO_4). **Guano bile-acid** possibly belongs to the glycocholic-acid group, and is found in Peruvian guano but has not been thoroughly studied.

Taurocholic Acid. This acid, which is found in the bile of man, carnivora, oxen and a few other herbivora, such as sheep and goats, has the constitution $C_{26}H_{45}NSO_7$. On boiling with acids and alkalies it splits into cholalic acid and taurin.

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[1] Zeitschr. f. physiol. Chem., Bdd. 12 and 13.

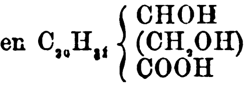
Taurocholic acid may be obtained, though only with difficulty, in fine needles which deliquesce in the air (PARKE[1]). It is very soluble in water, and can hold the difficultly soluble glycocholic acid in solution. This is the reason why a mixture of glycocholate with a sufficient quantity of taurocholate, which often occurs in ox-bile, is not precipitated by a dilute acid. Taurocholic acid is readily soluble in alcohol, but insoluble in ether. Its solutions have a bitter-sweet taste. Its salts are, as a rule, readily soluble in water, and the solutions of the alkali salts are not precipitated by copper sulphate, silver nitrate, or sugar of lead. Basic lead acetate gives, on the contrary, a precipitate which is soluble in boiling alcohol.

Taurocholic acid is best prepared from decolorized, crystallized dog-bile, which contains only taurocholate. The solution of this bile is precipitated by basic lead acetate and ammonia, and the washed precipitate dissolved in boiling alcohol. The filtrate is now treated with H_2S, and this filtrate is evaporated at a gentle heat to a small volume, and treated with an excess of water-free ether. The acid sometimes partially crystallizes.

Cheno-taurocholic Acid. This is the most essential acid of goose-bile and has the formula $C_{29}H_{49}NSO_6$. This acid, though little studied, is amorphous and soluble in water and alcohol.

As repeatedly mentioned above, the two bile-acids split on boiling with acids or alkalies into non-nitrogenous cholalic acid and glycocoll or taurin. Therefore we will now describe the products of this cleavage.

Cholalic Acid. The ordinary cholalic acid obtained as a decomposition product of human and ox bile, which occurs regularly in the contents of the intestine and in the urine in icterus, has, according to STRECKER[2] and nearly all recent investigators, the constitution $C_{24}H_{40}O_5$. According to MYLIUS,[3] cholalic acid is a monobasic alcohol-acid with a secondary and two primary alcohol groups. Its formula may therefore be written



$$C_{20}H_{31}\left\{\begin{array}{l}CHOH\\(CH_2OH)_2\\COOH\end{array}\right.$$

. On oxidation it first yields *dehydrocholalic acid* (AUTHOR)[1], and then *bilianic acid* (CLEVE).[2] The formulæ of these acids (when we take C_{24} for the cholalic acid) are $C_{24}H_{34}O_5$ and $C_{24}H_{34}O_8$. On reduction (in putrefaction) cholalic acid may yield *desoxycholalic* acid, $C_{24}H_{40}O_4$ (MYLIUS).[3]

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[1] Hoppe-Seyler, Med. chem. Untersuch., S. 160.

[2] The important investigations of Strecker on the bile-acids may be found in Ann. d. Chem. u. Pharm., Bdd. 65, 67, and 70.

[3] Ber. d. deutsch. chem. Gesellsch., Bd. 19, pp. 369–379 and 2000–2009.

Cholalic acid crystallizes partly with one molecule of water, in rhombic plates or prisms, and partly in larger rhombic tetrahedra or octahedra with 1 mol. of alcohol of crystallization (MYLIUS). These crystals become quickly opaque and porcelain-white in the air. They are quite insoluble in water (in 4000 parts cold and 750 parts boiling), rather soluble in alcohol, but soluble with difficulty in ether. The amorphous cholalic acid is less insoluble. The solutions have a bitter-sweetish taste. The crystals lose their alcohol of crystallization only after a lengthy heating to 100–120° C. The acid free from water and alcohol melts at + 195° C.

The alkali salts are readily soluble in water, but when treated with a concentrated caustic or carbonated alkali solution may be separated as an oily mass which becomes crystalline on cooling. The alkali salts are not readily soluble in alcohol, and on the evaporation of the alcohol they may crystallize. The specific rotatory power of the sodium salt is $(\alpha)D = +31°.4$. The watery solution of the alkali salts, when not too dilute, is precipitated immediately or after some time by sugar of lead or by barium chloride. The barium salt crystallizes in fine, silky needles, and it is rather insoluble in cold, but somewhat easily soluble in warm water. The barium salt, as well as the lead salt which is insoluble in water, is soluble in warm alcohol.

Cholalic acid is best prepared from ox-bile by the following method as suggested by MYLIUS:[4] Boil the bile for 24 hours with 5 parts its weight of a 30% caustic-soda solution, replacing the water lost by evaporation. Now saturate the liquid with CO_2 and evaporate nearly to dryness. The residue is extracted with 96% alcohol, and this alcoholic extract diluted with water until it contains at the most 20% alcohol, and completely precipitated with a $BaCl_2$ solution. The precipitate, which contains besides fatty acids also the choleic acid, is filtered and the cholalic acid precipitated from the filtrate by hydrochloric acid. After the cholalic acid has gradually crystallized out it is repeatedly recrystallized from alcohol or methyl alcohol.

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[1] Ber. d. deutsch. chem. Gesellsch., Bd. 14, S. 71.

[2] Bull. Soc. Chim., Tome 35.

[3] L. c.

[4] Zeitschr. f. physiol. Chem., Bd. 12.

Choleic Acid is another cholalic acid with the formula $C_{24}H_{40}O_4$ (LASSAR-COHN[1]) named by LATSCHINOFF.[2] This acid, which occurs in varying but always small quantities in ox-bile, is probably identical with desoxycholalic acid. Choleic acid first yields *dehydrocholeic acid*, $C_{24}H_{34}O_4$, and then *cholanic acid*, $C_{24}H_{34}O_8$, on oxidation.

Choleic acid may be obtained from the above-mentioned barium precipitate by first converting the barium salts into sodium salts by sodium carbonate and then fractionally precipitating the fatty acids by barium acetate and separating the choleic acid from the filtrate by hydrochloric acid and recrystallizing several times from glacial acetic acid.

Fellic Acid, $C_{23}H_{40}O_4$, is a cholalic acid, so called by SCHOTTEN,[3] and which he obtained from human bile, along with the ordinary acid. This acid is crystalline, is insoluble in water, and yields barium and magnesium salts which are very insoluble. It does not give PETTENKOFER'S reaction easily and gives a more reddish-blue color.

The conjugate acids of human bile have not been investigated. To all appearance human bile contains under different circumstances various conjugate bile-acids. In some cases the bile-salts of human bile are precipitated by $BaCl_2$, and in others not. According to the latest statements of LASSAR-COHN[4] three cholalic acids may be prepared from human bile, namely, ordinary CHOLALIC ACID, CHOLEIC ACID, and FELLIC ACID.

The hyo-glycocholic and cheno-taurocholic acids as well as the glycocholic acid of the bile of rodents yield corresponding cholalic acids.

On boiling with acids, on putrefaction in the intestine, or on heating, cholalic acids lose water and are converted into an anhydride, the so-called *dyslysin*. The dyslysin, $C_{24}H_{36}O_3$, corresponding to ordinary cholalic acid, and which occurs in fæces, is amorphous, insoluble in water and alkalies. *Choloidic acid*, $C_{24}H_{38}O_4$, is called the first anhydride or an intermediate product in the formation of dyslysin. On boiling dyslysin with caustic alkali it is reconverted into the corresponding cholalic acid.

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[1] Zeitschr. f. physiol. Chem., Bd. 17, S. 606.

[2] Ber. d. deutsch. chem. Gesellsch., Bdd. 18 and 20.

[3] *Ibid.*, Bd. 11, S. 268.

[4] Ber. d. deutsch. chem. Gesellsch., Bd. 27, S. 1339.

Glycocoll, $C_2H_5NO_2$, or amido-acetic acid, $NH_2.CH_2.COOH$, also called glycin, or sugar of gelatin, has been found in the muscles of *pecten irradians*, but it is of special interest as a decomposition product of certain protein substances—gelatin and spongin—as also of hippuric acid or glycocholic acid on splitting them by boiling with acids.

Glycocoll forms colorless, often large, hard rhombic crystals or four-sided prisms. The crystals taste sweet and dissolve easily in cold (4.3 parts) water. They are insoluble in alcohol and ether; in warm spirits of wine they dissolve, but with difficulty. Glycocoll combines with acids and bases. Under the last-mentioned combinations we must mention those with copper and silver. Glycocoll dissolves copper hydroxide in alkaline liquids, but does not reduce it at the boiling temperature. A boiling-hot solution of glycocoll dissolves freshly precipitated copper hydroxide, forming a blue liquid from which dark-blue needles crystallize on cooling, if the liquid is sufficiently concentrated. The combination of glycocoll with HCl is soluble in water and alcohol.

Glycocoll is best prepared from hippuric acid by boiling it 10–12 hours with 4 parts of dilute sulphuric acid, 1 : 6. After cooling separate the benzoic acid, concentrate the filtrate, remove the remainder of the benzoic acid by shaking with ether, remove the sulphuric acid by $BaCO_3$, and evaporate the filtrate to crystallization. In the preparation and quantitative estimation of glycocoll from gelatin we can proceed according to GONNERMANN's[1] modification of CH. FISCHER's[2] method. The gelatin is decomposed by sulphuric acid, the sulphuric acid removed by lead carbonate, the glycocoll transformed into hippuric acid by benzoyl chloride and caustic soda. This solution is acidified with sulphuric acid, extracted with acetic ether, and the syrupy residue of acetic ether dissolved in chloroform containing benzol. The precipitated hippuric acid after 24 hours is collected on a filter and first washed with chloroform containing benzol and then with pure chloroform.

Taurin, $C_2H_7NSO_3$, or amido-ethylsulphonic acid, $NH_2C_2H_4.SO_2OH$. This body is well known as a splitting product of taurocholic acid, and may occur in small quantities in the contents of the intestine. It has also been found in the lungs and kidneys of oxen and in the blood and muscles of cold-blooded animals.

Taurin crystallizes in colorless, often in large, shining, 4–6-sided prisms. It dissolves in 15–16 parts of water at ordinary temperatures, but rather more easily in warm water. It is insoluble in absolute alcohol and ether; in cold spirits of wine it dissolves slightly, but more when warm. Taurin yields acetic and sulphurous acids, but no alkali sulphides, on boiling with strong caustic alkali. The amount of sulphur can be determined as sulphuric acid after fusing with saltpetre and soda. Taurin combines with metallic oxides. The combination with mercuric oxide is white, insoluble, and is formed when a solution of taurin is boiled with freshly precipitated mercuric oxide (J. LANG[1]). This combination may be used in detecting the presence of taurin. Taurin is not precipitated by metallic salts.

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[1] Pflüger's Arch., Bd. 59.

[2] Zeitschr. f. physiol. Chem., Bd 19.

The preparation of taurin from bile is very simple. The bile is boiled a few hours with hydrochloric acid. The filtrate from the dyslysin and choloidic acid is concentrated well in the water-bath, and filtered so as to remove the common salt and other substances which have separated. Then evaporate to dryness, and treat the residue with strong alcohol, which dissolves the hydrochlorate of glycocoll, while the taurin remains. (The alcoholic solution of hydrochlorate of glycocoll may be used in the preparation of glycocoll by evaporating the alcohol and dissolving the residue in water, decomposing the solution with lead hydroxide, filtering, and freeing the solution from lead by H_2S, and strongly concentrating this filtrate. The crystals which separate are dissolved and decolorized by animal charcoal, and the solution evaporated to crystallization.) The above-obtained residue containing the taurin is dissolved in as little water as possible, filtered warm, and treated with an excess of alcohol. The crystalline precipitate which immediately forms is filtered as soon as possible, and the taurin now separates, on cooling, in very long needles or prisms. These crystals may be purified by recrystallization from a little warm water.

Though the taurin shows no positive reactions, it is chiefly identified by its crystalline form, by its solubility in water and insolubility in alcohol, by its combination with mercuric oxide, by its non-precipitability by metallic salts, and above all by its containing sulphur.

THE DETECTION OF BILE-ACIDS IN ANIMAL FLUIDS. To obtain the bile-acids pure so that PETTENKOFER's test can be applied to them, the proteid and fat must first be removed. The proteid is removed by making the liquid first neutral and then adding a great excess of alcohol, so that the mixture contains at least 85 vols. per cent of water-free alcohol. Now filter, extract the precipitated proteid with fresh alcohol, unite all filtrates, distil the alcohol, and evaporate to dryness. The residue is completely exhausted with strong alcohol, filtered, and the alcohol entirely evaporated from the filtrate. The new residue is dissolved in water, and filtered if necessary, and the solution precipitated by basic lead acetate and ammonia. The washed precipitate is dissolved in boiling alcohol, filtered while warm, and a few drops of soda solution added. Then evaporate to dryness, extract the residue with absolute alcohol, filter, and add an excess of ether. The precipitate now formed may be used for PETTENKOFER's test. It is not necessary to wait for a crystallization; but one must not consider the crystals which form in the liquid as being positively crystallized bile. It is also possible for needles of alkali acetate to be formed. For the detection of bile-acids in urine see Chapter XV.

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[1] See Maly's Jahresber., Bd. 6, S. 73.

Bile-pigments. The bile-coloring matters known thus far are relatively numerous, and in all probability there are still more. Most of the known bile-pigments are not found in the normal bile, but occur either in post-mortem bile or, principally, in the bile concrements. The pigments which occur under physiological conditions are the reddish-yellow *bilirubin*, the green *biliverdin*, and sometimes there is also observed in fresh human bile a pigment closely allied to *hydrobilirubin*. The pigments found in gall-stones are (besides the *bilirubin* and *biliverdin*) *bilifuscin*, *biliprasin*, *bilihumin*, *bilicyanin* (and *choletelin?*). Besides these, others have been observed in human and animal bile. The two above-mentioned physiological pigments, bilirubin and biliverdin, are those which serve to give the golden-yellow or orange-yellow or sometimes greenish color to the bile, or when, as is most frequently the case in ox-bile, the two pigments are present in the bile at the same time, producing the different shades between reddish brown and green.

Bilirubin. This pigment, according to the common acceptation, has the formula $C_{16}H_{18}N_2O_3$ (MALY[1]) and is designated by the names CHOLEPYRRHIN, BILIPHÆIN, BILIFULVIN, and HÆMATOIDIN. It occurs chiefly in the gall-stones as bilirubin-calcium. It occurs in the liver-bile of all vertebrates and in the bladder-bile especially in man and carnivora; sometimes, however, the latter when fasting or in a starving condition may have a green bile. It occurs also in the contents of the small intestine, in blood-serum of the horse, in old blood extravasations (as hæmatoidin), and in the urine and the yellow-colored tissue in icterus. Bilirubin is derived in all probability from hæmatin, which it closely resembles. It is converted into *hydrobilirubin*, $C_{32}H_{40}N_4O_7$ (Maly[1]) by hydrogen in a nascent state. It is claimed by several investigators to be identical with the urinary pigment *urobilin*, as well as with *stercobilin* (Masius and Vanlair[2]), which is found in the contents of the intestine. There is no doubt that a great similarity exists between these pigments, but their identity is emphatically denied by MacMunn.[3] On oxidation bilirubin yields biliverdin and other coloring matters (see below).

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[1] Wien. Sitzungsber., Bdd. 57 and 70.

Bilirubin is partly amorphous and partly crystalline. The amorphous bilirubin is a reddish-yellow powder of nearly the same color as amorphous antimony sulphide; the crystalline bilirubin has nearly the same color as crystallized chromic acid. The crystals, which can easily be obtained by allowing a solution of bilirubin in chloroform to spontaneously evaporate, are reddish-yellow, rhombic plates, whose obtuse angles are often rounded.

Bilirubin is insoluble in water, slightly soluble in ether, somewhat more soluble in alcohol, easily soluble in chloroform, especially in the warmth, and less soluble in benzol, carbon disulphide, amyl alcohol, fatty oils, and glycerin. Its solutions show no absorption-bands, but only a continuous absorption from the red to the violet end of the spectrum, and they have, even on diluting greatly, (1 : 500000) in a layer 1.5 c.cm. thick a decided yellow color. If a dilute solution of bilirubin in water is treated with an excess of ammonia and then with a zinc-chloride solution, the liquid is first colored deep orange and then gradually olive-brown and then green. This solution first gives a darkening of the violet and blue part of the spectrum and then the bands of alkaline cholecyanin (see below), or at least the bands of the pigment in the red between *C* and *D* close to *C*. This is a good reaction for bilirubin. The combinations of bilirubin with alkalies are insoluble in chloroform, and bilirubin may be separated from its solution in chloroform by shaking with dilute caustic alkali (differing from lutein). Solutions of bilirubin-alkali in water are precipitated by the soluble salts of the alkaline earths and also by metallic salts.

If an alkaline solution of bilirubin be allowed to stand in contact with the air, it gradually absorbs oxygen and green biliverdin is formed. Biliverdin is also formed from bilirubin by oxidation under other conditions. A green coloring matter similar in appearance is formed by the action of other reagents such as Cl, Br, and I. In these cases it does not seem to be biliverdin, but a substitution product of bilirubin (THUDICHUM,[1] MALY[2]), which is obtained.

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[1] Ann. d. Chem., Bd. 163.

[2] Centralbl f. d. med. Wissensch., 1871, S. 369.

[3] Journal of Physiol., Vol. 10, p. 71.

GMELIN'S *Reaction for Bile-pigments.* If we carefully pour under a solution of bilirubin-alkali in water nitric acid containing some nitrous acid, we obtain a series of colored layers at the juncture of the two liquids, in the following order from above downwards: green, blue, violet, red, and reddish yellow. This color reaction, GMELIN'S test, is very delicate and serves to detect the presence of one part bilirubin in 80,000 parts liquid. The green ring must never be absent; and also the reddish violet must be present at the same time, otherwise the reaction may be confused with that for lutein, which gives a blue or greenish ring. The nitric acid must not contain too much nitrous acid, for then the reaction takes place too quickly and it does not become typical. Alcohol must not be present in the liquid, because, as is well known, it gives a play of colors, in green or blue, with the acid.

HUPPERT'S *Reaction.* If a solution of bilirubin-alkali is treated with milk of lime or with calcium chloride and ammonia, a precipitate is produced consisting of bilirubin-calcium. If this moist precipitate, which has been washed with water, is placed in a test-tube and the tube half filled with alcohol which has been acidified with sulphuric acid, and heated to boiling for some time, the liquid becomes emerald-green or bluish green in color. This reaction is a good and easily performed test for bile-pigments.

In regard to the modifications of GMELIN'S test and certain other reactions for bile-pigments, see Chapter XV (Urine).

That the characteristic play of colors in GMELIN'S test is the result of an oxidation is generally admitted. The first oxidation step is the green biliverdin. Then follows a blue coloring matter which HEINSIUS and CAMPBELL[3] call *bilicyanin* and STOKVIS[4] calls *cholecyanin*, and which shows a characteristic absorption-spectrum. The neutral solutions of this coloring matter are, according to STOKVIS, bluish green or steel-blue with a beautiful blue fluorescence. The alkaline solutions are green and have no marked fluorescence. The neutral and alkaline solutions show three absorption-bands, one sharp and dark in the red between *C* and *D*, nearer to *C*; a second, less defined, covering *D*; and a third, forming only a faint shadow, in the green, exactly in the middle, between *D* and *E*. The strongly acid solutions are violet-blue and show two bands, described by Jaffé, between the lines *C* and *E*, separated from each other by a narrow space near *D*. The next oxidation step after these blue coloring matters is a red pigment, and lastly a yellowish-brown pigment, called *choletelin* by Maly,[1] which in neutral alcoholic solutions does not give any absorption spectrum, but in acid solution gives a band between *b* and *F*.

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[1] Journal of the Chem Soc., (2) Vol. 13.

[2] Wien. Sitzungsber., Bd. 72.

[3] Pflüger's Arch., Bd. 4, S. 529.

[4] Centralbl. f. d. med. Wissensch., 1872, S. 785.

Bilirubin is best prepared from gall-stones of oxen, these concretions being very rich in bilirubin-calcium. The finely powdered concrement is first exhausted with ether and then with boiling water, so as to remove the cholesterin and bile-acids. The powder is then treated with hydrochloric acid, which sets free the pigment. Wash thoroughly with water and alcohol, dry, and extract repeatedly with boiling chloroform. After distilling the chloroform from the solution, treat the powdered residue with absolute alcohol to remove the bilifuscin; dissolve the remaining bilirubin in a little chloroform; precipitate it from this solution by alcohol, and do this several times if necessary. The bilirubin is finally dissolved in boiling chloroform and allowed to crystallize on cooling. The quantitative estimation of bilirubin may be made by the spectrophotometrical method, according to the steps suggested for the blood-coloring matters.

Biliverdin, $C_{16}H_{18}N_2O_4$. This body, which is formed by the oxidation of bilirubin, occurs in the bile of many animals, in vomited matter, in the placenta of the bitch (?), in the shells of birds' eggs, in the urine in icterus, and sometimes in gall-stones, although in very small quantities.

Biliverdin is amorphous, or at least it has not been obtained in well-defined crystals. It is insoluble in water, ether, and chloroform (this is true at least for the artificially prepared biliverdin, while the green pigment of ox-bile is soluble in chloroform, according to MacMunn[2]), but is soluble in alcohol or glacial acetic acid, showing a beautiful green color. It is dissolved by alkalies, giving a brownish-green color, and this solution is precipitated by acids, as well as by calcium, barium, and lead salts. Biliverdin gives HUPPERT's and GMELIN's reactions, commencing with the blue color. It is converted into hydrobilirubin by nascent hydrogen. On allowing the green bile to stand, also by the action of ammonium sulphide, the biliverdin may be reduced to bilirubin (HAYCRAFT and SCOFIELD[1]).

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[1] Wien. Sitzungsber., Bd. 59. See also Jaffé, Centralbl. f. d. med. Wissensch., 1868, and Heinsius and Campbell, Pflüger's Arch., Bd. 4.

[2] Journal of Physiol., Vol. 6.

Biliverdin is most simply prepared by allowing a thin layer of an alkaline solution of bilirubin to stand exposed to the air in a dish until the color is brownish green. The solution is then precipitated by hydrochloric acid, the precipitate washed with water until no HCl reaction is obtained, then dissolved in alcohol and the pigment again separated by the addition of water. Any bilirubin present may be removed by means of chloroform.

Bilifuscin, so named by STÄDELER[2], is an amorphous brown pigment, soluble in alcohol and alkalies, nearly insoluble in water and ether, and soluble with great difficulty in chloroform (when bilirubin is not present at the same time). When pure bilifuscin does not give GMELIN'S reaction. It is found in post-mortem bile and gall-stones. *Biliprasin* is a green pigment prepared by STÄDELER from gall-stones, which perhaps is only a mixture of biliverdin and bilirubin. *Bilihumin* is the name given by STÄDELER to that brownish amorphous residue which is left after extracting gall-stones with chloroform, alcohol, and ether. It does not give GMELIN'S test. *Bilicyanin* is also found in human gall-stones (HEINSIUS and CAMPBELL). *Cholohæmatin*, so called by MACMUNN,[3] is a pigment often occurring in sheep- and ox-bile and characterized by four absorption-bands, and which is formed from hæmatin by the action of sodium amalgam. In the dried condition obtained by the evaporation of the chloroform solution it is green, and in alcoholic solution olive-brown.

GMELIN'S and HUPPERT'S reactions are generally used to detect the presence of bile-pigments in animal fluids or tissues. The first, as a rule, can be performed directly, and the presence of proteid does not interfere with it, but, on the contrary, it brings out the play of colors more strikingly. If blood-coloring matters are present at the same time, the bile-coloring matters are first precipitated by the addition of sodium phosphate and milk of lime. This precipitate containing the bile-pigments may be used directly in HUPPERT'S reaction, or may be treated with water and some hydrochloric acid, and then shaken with chloroform free from alcohol, and this chloroform solution used in testing for the bile-pigments.

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[1] Centralbl. f. Physiol., 1889, S. 222, and Zeitschr. f. physiol. Chem., Bd. 14.

[2] Vierteljahrschr. d. naturf. Gesellsch. in Zürich, Bd. 8, cited from Hoppe-Seyler, Physiol. u. path. chem. Analyse, 6. Aufl., S. 225.

[3] Journal of Physiol., Vol. 6.

Bilirubin is detected in blood, according to HEDENIUS,[1] by precipitating the proteins by alcohol, filtering and acidifying the filtrate with hydrochloric or sulphuric acid, and boiling. The liquid becomes of a greenish color. Serum and serous fluids may be boiled directly with a little acid after the addition of alcohol.

Besides the bile-acids and bile-pigments we also have in the bile *cholesterin*, *lecithin*, *palmitin*, *stearin*, *olein*, and *soaps* of the corresponding *fatty acids*. LASSAR-COHN[2] has also found *myristic acid* in ox-bile. In some animals the bile contains a *diastatic enzyme*. *Cholin* and *glycero-phosphoric acid*, when they are present, may be considered as decomposition products of lecithin. *Urea* occurs, though only as traces, as a physiological constituent of human, ox, and dog bile. Urea occurs in the bile of the shark and ray in such large quantities that it forms one of the chief constituents of the bile.[3] The *mineral constituents* of the bile are, besides the alkalies, to which the bile acids are united, sodium and potassium chloride, calcium and magnesium phosphate, and iron—0.04–0.115 p. m. in human bile, chiefly combined with phosphoric acid (YOUNG[4]). Traces of copper are habitually present, and traces of zinc are often found. Sulphates are entirely absent or only occur in very small amounts.

The quantity of iron in the bile varies very much. According to NOVI[5] it is dependent upon the kind of food, and in dogs it is lowest with a bread diet and highest with a meat diet. According to DASTRE[6] this is not the case. The quantity of iron in the bile varies even though a constant diet is kept up, and the variation is dependent upon the formation and destruction of blood. The question as to the extent of elimination by the bile of the iron introduced into the body has received various answers. There is no doubt that the liver has the property of collecting and retaining iron and also other metals from the blood. Certain investigators, such as NOVI and KUNKEL,[7] are of the opinion that the introduced and transitorily retained iron in the liver is eliminated by the bile, while others, such as HAMBURGER,[1] GOTTLIEB,[2] and ANSELM,[3] deny any such elimination of iron by the bile.

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[1] Upsala Läkaref. Förh., Bd. 29.

[2] Zeitschr. f. physiol. Chem., Bd. 17.

[3] Investigation not published by the author.

[4] Journal of Anat. and Physiol., Vol. 5, p. 158.

[5] See Maly's Jahresber., Bd. 20, S. 273.

[6] Arch. de Physiol., (5) Tome 3.

[7] Pflüger's Arch., Bd. 14.

Quantitative Composition of the Bile. Complete analyses of human bile have been made by HOPPE-SEYLER and his pupils. The bile was removed as fresh as possible from the gall-bladder of cadavers whose livers showed no remarkable change. The following figures of SOCOLOFF[4] are the average of six analyses, and those of HOPPE-SEYLER[5] of five analyses. The relationship between the glycocholate and taurocholate was found by fusing the precipitate, consisting of biliary alkalies obtained by ether from the alcoholic extract, with saltpetre and soda. On determining the amount of sulphur in the fused mass the taurocholic acid can be calculated from this. 100 parts $BaSO_4$ correspond to 220.86 parts taurocholic acid. The figures are parts per 1000.

	TRIFANOWSKI.[6]		SOCOLOFF.	HOPPE-SEYLER.
	I.	II.		
Mucin	24.8	13.0	37.20	12.9
Remaining bodies insol. in alcohol.	4.5	14.6		1.4
Taurocholate	7.5	19.2	15.67	8.7
Glycocholate	21.0	4.4	49.04	30.3
Soaps	8.1	16.3	14.60	13.9
Cholesterin	2.5	3.3	.....	3.5
Lecithin	5.2	0.2	.....	5.3
Fat		3.6	.....	7.3
Ferric phosphate	..	..	....	0.166

Older and less complete analyses of human bile have been made by FRERICHS and v. GORUP-BESANEZ. The bile analyzed by them was from perfectly healthy persons who had been executed or accidentally killed. The two analyses of FRERICHS are, respectively, of (I) an 18-year-old and (II) a 22-year-old male. The analyses of v. GORUP-BESANEZ are of (I) a man of 49 and (II) a woman of 29. The results are, as usual, in parts per 1000.

	FRERICHS.[7]		v. GORUP-BESANEZ.[8]	
	I.	II.	I.	II.
Water	860.0	859.2	822.7	898.1
Solids	140.0	140.8	177.3	101.9
Biliary salts	72.2	91.4	107.9	56.5
Mucus and pigments	26.6	29.8	22.1	14.5
Cholesterin	1.6	2.6	47.3	30.9
Fat	3.2	9.2		
Inorganic substances	6.5	7.7	10.8	6.2

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[1] Zeitschr. f. physiol. Chem., Bdd. 2 and 4.

[2] *Ibid.*, Bd. 15.

[3] Ueber die Eisenausscheidung der Galle. Inaug. Diss. Dorpat, 1891.

[4] Pflüger's Arch., Bd. 12.

[5] Physiol. Chem., S. 301.

[6] Pflüger's Arch., Bd. 9.

[7] Cit. from Hoppe-Seyler's Physiol. Chem., S. 299.

[8] *Ibid.*

Human liver-bile is poorer in solids than the bladder-bile. In several cases it only contained 12–18 p. m. solids, but the bile in these cases is hardly to be considered as normal. JACOBSEN[1] found 22.4–22.8 p. m. solids in a specimen of bile. The AUTHOR,[2] who had occasion to analyze the liver-bile in seven cases of biliary fistula, has repeatedly found 25–28 p. m. solids. In a case of a corpulent woman the quantity of solids in the liver-bile varied between 30.10–38.6 p. m. in ten days.

Human bile sometimes, but not always, contains sulphur in an ethereal sulphuric-acid combination. The quantity of such sulphur may even amount to ¼–⅓ of the total sulphur. Human bile is habitually richer in glycocholic than in taurocholic acid. In six cases of liver-bile analyzed by the AUTHOR the relationship of taurocholic to glycocholic acid varied between 1 : 2.07 and 1 : 14.36. The bile analyzed by JACOBSEN contained no taurocholic acid.

As example of the composition of human liver-bile we give the following results of three analyses made by the AUTHOR.[3] The results are calculated in parts per 1000.

Solids	25.200	35.260	25.400
Water	974 800	964.740	974.600
Mucin and pigments	5.290	4.290	5.150
Bile-salts	9.810	18.240	9.040
Taurocholate	3.034	2.079	2.180
Glycocholate	6.276	16.161	6.860
Fatty acids from soaps	1.230	1.360	1.010
Cholesterin	0.630	1.600	1 500
Lecithin	0.220	0.574	0.650
Fat		0.956	0.610
Soluble salts	8.070	6.760	7.250
Insoluble salts	0.250	0.490	0.210

BAGINSKY and SOMMERFELD[4] have found true mucin, mixed with some nucleoalbumin, in the bladder-bile of children. The bile contained on an average 896.5 p. m. water; 103.5 p. m. solids; 20 p. m. mucin; 9.1 p. m. mineral substances; 25.2 p. m. bile-salts, of which 16.3 p. m. were glycocholate and 8.9 p. m. taurocholate; 3.4 p. m. cholesterin; 6.7 p. m. fat, and 2.8 p. m. leucin.

Amongst the mineral constituents the chlorine and sodium occur to the greatest extent. The relationship between potassium and sodium varies considerably in different biles. Sulphuric acid and phosphoric acid only occur in very small quantities. The quantity of iron in the liver-bile in three cases investigated by the AUTHOR was 0.018–0.044 p. m., calculated on the fresh bile.

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[1] Ber. d. deutsch. chem. Gesellsch., Bd. 6.

[2] Nova Acta Reg Soc. Scient. Upsala, Bd. 16.

[3] L. c.

[4] Verhandl d. physiol. Gesellsch. zu Berlin, 1894–95, Nos. 13, 14, 15.

The quantity of pigment in human bile is, according to NOEL-PATON,[1] 0.4–1.3 p. m. for a case of biliary fistula. The method used in determining the pigments in this case was not quite trustworthy. More exact results obtained by spectro-photometric methods are on record for dogs' bile. According to STADELMANN[2] dogs' bile contains on an average 0.6–0.7 p. m. bilirubin. At the most, only 7 milligrams pigment are secreted per kilo of body in the 24 hours.

In animals the relative proportion of the two acids varies very much. It has been found, on determining the amount of sulphur, that, so far as the experiments have gone, taurocholic acid is the prevailing acid in carnivorous mammalia, birds, snakes, and fishes. Among the herbivora sheep and goats have a predominance of taurocholic acid in the bile. Ox-bile sometimes contains taurocholic acid in excess, in other cases glycocholic acid predominates, and in a few cases the latter occurs almost alone. The bile of the rabbit, hare, and kangaroo contains, like the bile of the pig, almost exclusively glycocholic acid. A distinct influence on the relative amounts of the two bile-acids by different foods has not been detected. RITTER[3] claims to have found a decrease in the quantity of taurocholic acid in calves when they pass from the milk to the plant diet.

In the above-mentioned calculation of the taurocholic acid from the quantity of sulphur in the bile-salts it must be remarked that no exact conclusion can be drawn from this calculation as long as we have not investigated whether other kinds of bile contain sulphur in combinations other than taurocholic acid, as in human and shark bile.

The *gases* of the bile consist of a large quantity of carbon dioxide, which increases with the amount of alkalies, only traces of oxygen, and a very small quantity of nitrogen.

Little is known in regard to the *properties of the bile in disease.* The quantity of *urea* is found to be considerably increased in uræmia. *Leucin* and *tyrosin* are observed in acute yellow atrophy of the liver and in typhus. Traces of *albumin* (without regard to nucleoalbumin) have several times been found in the human bile. The so-called *pigmentary acholia*, or the secretion of a bile containing bile-acids but no bile-pigments, has also been repeatedly noticed. In all such cases observed by RITTER[1] he found a fatty degeneration of the liver-cells, in return for which, even in excessive fat infiltration, a normal bile containing pigments was secreted. The secretion of a bile nearly free from bile-acids has been observed by HOPPE-SEYLER[2] in amyloid degeneration of the liver. In animals, dogs, and especially rabbits it has been observed that the blood-pigments pass into the bile in poisoning and in other cases, causing a destruction of the blood-corpuscles, as also after intravenous hæmoglobin injection (WERTHEIMER and MEYER,[3] FILEHNE,[4] STERN[5]).

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[1] Rep. Lab. Roy. Soc. Coll. Phys. Edinb., Vol. 3.

[2] Der Icterus, etc. Stuttgart, 1891.

[3] Cit. from Maly's Jahresber., Bd. 6, S. 195.

Chemical Formation of the Bile. The first question to be answered is the following: Do the specific constituents of the bile, the bile-acids and bile-pigments, originate in the liver; and if this is the case, do they come from this organ only, or are they also formed elsewhere?

The investigations of the blood, and especially the comparative investigations of the blood of the portal and hepatic veins under normal conditions, have not given any answer to this question. To decide this, therefore, it is necessary to extirpate the liver of animals or isolate it from the circulation. If the bile constituents are not formed in the liver, or at least not alone in this organ, but only eliminated from the blood, then, after the extirpation or removal of the liver from the circulation, an accumulation of the bile constituents is to be expected in the blood and tissues. If the bile constituents, on the contrary, are formed exclusively in the liver, then the above operation naturally would give no such result. If the choledochus duct is tied, then the bile constituents will be collected in the blood or tissues whether they are formed in the liver or elsewhere.

From these principles KÖBNER[6] has tried to demonstrate by experiments on frogs that the *bile-acids* are produced exclusively in the liver. While he was unable to detect any bile-acids in the blood and tissues of these animals after extirpation of the liver, still he was able to discover them on tying the choledochus duct. The investigations of LUDWIG and FLEISCHL[7] show that in the dog the bile-acids originate in the liver alone. After tying the choledochus duct they observed that the bile constituents were absorbed by the lymphatic vessels and passed into the blood through the thoracic duct. Bile-acids could be detected in the blood after such an operation, while they could not be detected in the normal blood. But when the choledochus and thoracic ducts were both tied at the same time, then not the least trace of bile-acids could be detected in the blood, while if they are also formed in other organs and tissues they should have been present.

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[1] Compt. rend., Tome 74, and Journ. de l'anat. et de la physiol., 1872.

[2] Physiol. Chem., S. 317.

[3] Compt. rend., Tome 108.

[4] Virchow's Arch., Bd. 121.

[5] *Ibid.*, Bd. 123.

[6] See Heidenhain, Physiologie der Absonderungsvorgänge in Hermann's Handbuch, Bd. 5.

[7] Arbeiten aus der physiol. Anstalt zu Leipzig, Jahrgang 9.

Other ways have been tried to demonstrate the formation of bile-acid in the liver-cells. ALEX. SCHMIDT and KALLMEYER[1] have shown that the isolated liver-cells, which have been washed with a physiological NaCl solution, have the property, in the presence of hæmoglobin and glycogen, of increasing the quantity twofold, of substances soluble in alcohol but insoluble in ether. This tends to show the formation of bile alkalies.

From older statements of CLOEZ and VULPIAN as well as VIRCHOW the bile-acids also occur in the suprarenal capsule. These statements have not been confirmed by later investigations of STADELMANN and BEIER.[2] At the present time we have no ground for supposing that the bile-acids are formed elsewhere than in the liver.

It has been indubitably proved that the *bile-pigments* may be formed in other organs besides the liver, for, as is generally admitted, the coloring matter hæmatoidin, which occurs in old blood extravasations, is identical with the bile-pigment bilirubin (see page 145). LATSCHENBERGER[3] has also observed in horses, under pathological conditions, a formation of bile-pigments from the blood-coloring matters in the tissues. Also the occurrence of bile-pigments in the placenta seems to depend on their formation in that organ, while the occurrence of small quantities of bile-pigments in the blood-serum of certain animals probably depends on an absorption of the same.

Although the bile-pigments may be formed in other organs besides the liver, still it is of first importance to know what bearing this organ has on the elimination and formation of bile-pigments.

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[1] Kallmeyer, Ueber die Entstehung der Gallensäuren, etc." Inaug. Diss. Dorpat, 1889.

[2] Zeitschr. f. physiol. Chem., Bd. 18. This contains the older literature.

[3] Maly's Jahresber., Bd. 16, S. 301, and Monatshefte f. Chem., Bd. 9.

In this regard it must be recalled that the liver is an excretory organ for the bile-pigments circulating in the blood. TARCHANOFF[1] has observed, in a dog with biliary fistula, that intravenous injection of bilirubin causes a very considerable increase in the bile-pigments eliminated. This statement has been confirmed lately by the investigations of VOSSIUS.[2]

Numerous experiments have been made to decide the question whether the bile-pigments are only eliminated by the liver or whether they are also formed therein. By experimenting on pigeons STERN[3] was able to detect bile-pigments in the blood-serum five hours after tying the biliary passages alone, while after tying all the vessels of the liver and also the biliary passages no bile-pigments could be detected either in the blood or the tissues of the animal, which was killed 10–12 hours after the operation. MINKOWSKI and NAUNYN[4] have also found that poisoning with arseniuretted hydrogen produces a liberal formation of bile-pigments and the secretion, after a short time, of a urine rich in biliverdin in previously healthy geese. In geese with extirpated livers this does not occur.

No such experiments can be carried out on mammalia, as they do not live long enough after the operation; still there is no doubt that this organ is the chief seat of the formation of bile-pigments under physiological conditions.

In regard to the materials from which the bile-acids are produced, it may be said with certainty that the two components, glycocoll and taurin, which are both nitrogenized, are formed from the protein bodies. In regard to the origin of the non-nitrogenized cholalic acid, which was formerly considered as originating from the fats, we know nothing positively.

The blood-coloring matters are considered as the mother-substance of the bile-pigments. If the identity of hæmatoidin and bilirubin was settled beyond a doubt, then this view might be considered as proved. Independently, however, of this identity, which is not admitted by all investigators, the view that the bile-pigments are derived from the blood-coloring matters has strong arguments in its favor. It has been shown by several experimenters that a yellow or yellowish-red pigment can be formed from the blood-coloring matters, which gives Gmelin's test, and which, though it may not form a complete bile-pigment, is at least a step in its formation (Latschenberger[1]). A further proof of the formation of the bile-pigments from the blood-coloring matters consists in the fact that hæmatin yields urobilin, which is identical with hydrobilirubin, on reduction (Hoppe-Seyler and others). Other investigators (Nencki and Sieber and Le Nobel[2]) claim that the substance thus obtained is not true urobilin, but, all things considered, it seems to be so very nearly related that this relationship can be considered as a proof of the formation of bilirubin from blood-pigments. Further, hæmatoporphyrin (see page 144) and bilirubin are isomers, according to Nencki and Sieber, and nearly allied. The formation of bilirubin from the blood-coloring matters is shown, according to the observations of several investigators,[3] by the appearance of free hæmoglobin in the plasma—produced by the destruction of the red corpuscles by widely differing influences (see below) or by the injection of hæmoglobin solution—causing an increased formation of bile-pigments. The amount of pigments in the bile is not only considerably increased, but the bile-pigments may even pass into the urine under certain circumstances (icterus). After the injection of hæmoglobin solution into a dog either subcutaneously or in the peritoneal cavity, Stadelmann and Gorodecki[3] observed in the secretion of pigments by the bile an increase of 61% which lasted for more than twenty-four hours.

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[1] Pflüger's Arch., Bd. 9.

[2] Cit. from Stadelmann, Der Icterus, etc.

[3] Arch. f. exp. Path. u. Pharm., Bd. 19.

[4] *Ibid.*, Bd. 21

If, then, iron-free bilirubin is derived from the hæmatin containing iron, then iron must be split off. This process may be represented by the following formula, according to Nencki and Sieber,[4]

$$C_{32}H_{32}N_4O_4Fe + 2H_2O - Fe = 2C_{16}H_{18}N_2O_3,$$

though in reality it is probably more complicated. The question in what form or combination the iron is split off is of special interest, and also whether it is eliminated by the bile. This latter does not seem to be the case. In 100 parts of bilirubin which are eliminated by the bile there are only 1.4–1.5 parts iron, according to **Kunkel**[1]; while 100 parts hæmatin contain about 9 parts iron. **Minkowski** and **Baserin**[2] have also found that the abundant formation of bile-pigments occurring in poisoning by arseniuretted hydrogen does not increase the quantity of iron in the bile. The quantity apparently does not correspond with that in the decomposed blood-coloring matters.

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[1] L. c.

[2] See Chapter VI on the blood, p. 144.

[3] See Stadelmann, Der Icterus, etc.

[4] Arch. f. exp. Path. u. Pharm., Bd. 24.

On the contrary, it seems as if the iron, at least for a time, is retained by the liver as a pigment rich in iron. Such a pigment containing iron, which was formed by the decomposition of hæmoglobin, was observed by **Naunyn** and **Minkowski**[3] in the livers of birds, in arseniuretted hydrogen icterus. **Latschenberger**[4] claims that a yellow or yellowish-red pigment, "*choleglobin*," is derived from the blood-coloring matters, and acts as a step in the formation of the bile-pigments; and besides this he mentions another body consisting of dark grains and containing iron, which he designates as *melanin*. **Neumann**[5] has observed in blood extravasations and thrombi, besides hæmatoidin, a pigment containing iron, for which he has proposed the name *hæmatosiderin*.

What relationship does the formation of bile-acids bear to the formation of bile-pigments? Are these two chief constituents of the bile derived simultaneously from the same material, and can we detect a certain connection between the formation of bilirubin and bile-acids in the liver? The investigations of **Stadelmann**[6] teach us that this is not the case. With increased formation of bile-pigments the bile-acids decrease and the supply of hæmoglobin to the liver acts in strongly increasing the formation of bilirubin, but simultaneously strongly decreases the production of bile-acids. According to **Stadelmann** the formation of bile-pigments and bile-acids is due to a special activity of the cells.

An absorption of bile from the liver by the lymphatic vessels and the passage of the bile constituents into the blood and urine occurs in retarded discharge of the bile, and usually in different forms of *hepatogenic icterus*. But bile-pigments may also pass into the urine under other circumstances, especially in animals where a solution or destruction of the red blood-corpuscles takes place through injection of water or a solution of biliary salts, through poisoning by ether, chloroform, arseniuretted hydrogen, phosphorus, or toluylendiamin; and in other cases. This occurs also in man in grave infectious diseases. We have therefore a second form of icterus, in which the blood-coloring matters are transformed into bile-pigments elsewhere than in the liver, namely, in the blood—a *hæmatogenic* or *anhepatogenic icterus.* The occurrence of a hæmatogenic icterus has been made very probable by the investigations of MINKOWSKI and NAUNYN, AFANASSIEW, SILBERMANN, and especially STADELMANN.[1] This statement has been proven in certain of the above-mentioned cases, as after poisoning with phosphorus, toluylendiamin, and arseniuretted hydrogen, by direct experiment.

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[1] Pflüger's Arch., Bd. 14, S. 353.

[2] Arch. f. exp. Path. u. Pharm., Bd. 23.

[3] L. c.

[4] *Ibid.*

[5] Virchow's Arch., Bd. 111.

[6] Der Icterus, etc.

The icterus is also in these cases heptogenic; it depends upon an absorption of bile-pigments from the liver, and this absorption seems to originate in the different cases in somewhat different ways. Thus the bile may be viscous and cause a stowing of the bile by counteracting the low secretion pressure. In other cases the fine biliary passages may be compressed by an abnormal swelling of the liver-cells, or a catarrh of the bile-passages may occur causing a stowage of the bile (STADELMANN). The other forms of so-called hæmatogenic icterus are now explained in an analogous way.

Bile Concretions.

The concrements which occur in the gall-bladder vary considerably in size, form, and number, and are of three kinds, depending upon the kind and nature of the bodies forming their chief mass. One group of gall-stones contains lime-pigment as chief constituent, the other cholesterin, and the third calcium carbonate and phosphate. The concrements of the last-mentioned group occur very seldom in man. The so-called cholesterin stones are those which occur most frequently in man, while the lime-pigment stones are not found very often in man, but often in oxen.

The *pigment-stones* are generally not large in man, but in oxen and pigs they are sometimes found the size of a walnut or even larger. In most cases they consist chiefly of bilirubin-calcium with little or no biliverdin. Sometimes also small black or greenish black, metallic-looking stones are found, which consist chiefly of bilifuscin along with biliverdin. Iron and copper seem to be regular constituents of pigment-stones. Manganese and zinc have also been found a few cases. The pigment-stones are generally heavier than water.

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[1] The literature belonging to this subject is found in Stadelmann, Der Icterus, etc. Stuttgart, 1891.

The *cholesterin-stones*, whose size, form, color, and structure may vary greatly, are often lighter than water. The fractured surface is radiated, crystalline, and frequently shows crystalline, concentric layers. The cleavage fracture is waxy in appearance, and the fractured surface when rubbed by the nail also becomes like wax. By rubbing against each other in the gall-bladder they often become faceted or take other remarkable shapes. Their surface is sometimes nearly white and waxlike, but generally their color is variable. They are sometimes smooth, in other cases they are rough or uneven. The quantity of cholesterin in the stones varies from 642–981 p. m. (RITTER[1]). The cholesterin-stones also sometimes contain variable amounts of lime-pigments which give them a very changeable appearance.

Cholesterin, $C_{26}H_{44}O$, or, according to OBERMÜLLER, $C_{27}H_{46}O$. Cholesterin is generally considered as a monatomic alcohol of the formula $C_{26}H_{43}.OH$. According to the investigations of OBERMÜLLER,[2] who has analyzed several cholesterin compounds, it seems that the formula is rather $C_{27}H_{45}OH$. It yields a colored hydrocarbon, cholesterilin, with concentrated sulphuric acid, and this hydrocarbon is claimed by WEYL[3] to be closely related to the terpene group. Cholesterin is also claimed to be closely allied to cholalic acid.

Cholesterin occurs in small amounts in nearly all animal fluids and juices. It occurs only rarely in the urine, and then in very small quantities. It is also found in the different tissues and organs—especially abundant in the brain and the nervous system, —further in the yolk of the egg, in semen, and in wool-fat (together with isocholesterin). It appears also in the contents of the intestine, in excrements, and in the meconium. It occurs pathologically especially in gall-stones, as well as in atheromatous cysts, in pus, in tuberculous masses, old transudations, cystic fluids, sputum, and tumors. Several kinds of cholesterin seem to occur in the plant world.

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[1] Journal de l'anat. et de la physiol., 1872.

[2] Du Bois-Reymond's Arch., 1889, and Zeitschr. f. physiol. Chem., Bd. 15.

[3] *Ibid.*, 1886, S. 182.

Cholesterin which crystallizes from warm alcohol on cooling, and that which is present in old transudations, contains 1 mol. of water of crystallization, melts at 145° C., and forms colorless, transparent plates whose sides and angles frequently appear broken and whose acute angle is often 76° 30′ or 87° 30′. In large quantities it appears as a mass of white plates which shine like mother-of-pearl and have a greasy feel.

Cholesterin is insoluble in water, dilute acids and alkalies. It is neither dissolved nor changed by boiling caustic alkali. It is easily soluble in boiling alcohol, and crystallizes on cooling. It dissolves readily in ether, chloroform, and benzol, and also in the volatile or fatty oils. It is dissolved to a slight extent by alkali salts of the bile-acids.

Among the many combinations of cholesterin studied by OBERMÜLLER[1] the propionic ester, $C_2H_5.CO.O.C_{27}H_{45}$, is of special interest. This is used in the detection of cholesterin. For the detection of cholesterin we make use of its reaction with concentrated sulphuric acid, which, as above stated, gives a colored hydrocarbon with this acid.

If a mixture of five parts sulphuric acid and one part water acts on a cholesterin crystal, this crystal will show colored rings, first a bright carmine-red and then violet. This fact is made use of in the microscopic detection of cholesterin. Another test, and one very good for the microscopical detection of cholesterin, consists in treating the crystals first with the above dilute acid and then with some iodine solution. The crystals will be gradually colored violet, bluish green, and a beautiful blue.

SALKOWSKI'S[2] *Reaction.*—The cholesterin is dissolved in chloroform and then treated with an equal volume of concentrated sulphuric acid. The cholesterin solution becomes first bluish red, then gradually more violet-red, while the sulphuric acid appears dark red with a greenish fluorescence. If the chloroform solution is poured into a porcelain dish it becomes violet, then green, and finally yellow.

LIEBERMANN-BURCHARD'S[3] *Reaction.*—Dissolve the cholesterin in about 2 c.c. chloroform and add first 10 drops acetic anhydride and then concentrated sulphuric acid drop by drop. The mixture will first be beautiful red, then blue, and finally, if not too much cholesterin or sulphuric acid is present, a permanent green. In the presence of very little cholesterin the green color may appear immediately.

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[1] L. c.

[2] Pflüger's Arch., Bd. 6.

[3] C. Liebermann, Ber. d. deutsch. chem. Gesellsch., Bd. 18, S. 1805. H. Burchard, Beiträge zur Kenntniss der Cholesterine. Rostock, 1889.

Pure, dry cholesterin when fused in a test-tube over a low flame with 2 to 3 drops propionic anhydride yields a mass which on cooling is first violet, then blue, green, orange, carmine red, and finally copper-red. It is best to re-fuse the mass on a glass rod and then to observe the rod on cooling, holding it against a dark background (OBERMÜLLER).[1]

SCHIFF'S *Reaction*. If a little cholesterin is placed in a porcelain dish with the addition of a few drops of a mixture of two to three vols. conc. hydrochloric acid or sulphuric acid and one vol. of a medium solution of ferric chloride, and carefully evaporated to dryness over a small flame, a reddish-violet residue is first obtained and then a bluish violet.

If a small quantity of cholesterin is evaporated to dryness with a drop of concentrated nitric acid, we obtain a yellow spot which becomes deep orange-red with ammonia or caustic soda (not a characteristic reaction).

Isocholesterin. This body, so called by SCHULZE,[2] is isomeric with the ordinary cholesterin and occurs in wool-fat, and is therefore found in abundant quantities in so-called lanolin. It does not give SALKOWSKI'S reaction. It melts at 138–138°.5.

We make use of the so-called cholesterin-stones in the preparation of cholesterin. The powder is first boiled with water and then repeatedly boiled with alcohol. The cholesterin which on cooling separates from the warm filtered solution is boiled with a solution of caustic potash in alcohol so as to saponify any fat. After the evaporation of the alcohol we extract the cholesterin from the residue with ether, by which the soaps are not dissolved, filter, evaporate the ether, and purify the cholesterin by recrystallization from alcohol-ether. The cholesterin may be extracted from tissues and fluids by first extracting with ether and then purifying as above.

It is detected and determined quantitatively in tissue, etc., by this same method. It is ordinarily easily detected in transudations and pathological formations by means of the microscope.

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[1] L. c.

[2] Ber. d. deutsch. chem. Gesellch., Bd. 6; Journal f. prakt. Chem., N. F. Bd. 25, S. 458; and Zeitschr. f. physiol. Chem., Bd. 14, S. 522. See also E. Schulze and J. Barbieri, Journal f. prakt. Chem., N. F. Bd. 25, S. 159.

CHAPTER IX.

DIGESTION.

THE purpose of the digestion is to separate those constituents of the food which serve as the nutriment of the body from those which are useless, and to separate each in such a form that it may be taken up by the blood from the alimentary canal and employed for the various purposes in the organism. This demands not only mechanical but also chemical action. The first action, which is essentially dependent upon the physical properties of the food, consists in a tearing, cutting, crushing, or grinding of the food, and serves chiefly to convert the nutritive bodies into a soluble and easily absorbed form, or in the splitting of the same into simpler combinations for use in the animal synthesis. The solution of the nutritive bodies may take place in certain cases by the aid of water alone, but in most cases a chemical metamorphosis or splitting is necessary, and is effected by means of the acid or alkaline fluids secreted by the glands. The study of the processes of digestion from a chemical standpoint must therefore begin with the digestive fluids, their qualitative and quantitative composition, as well as their action on the nutriments and foods.

I. The Salivary Glands and the Saliva.

The **salivary glands** are partly *albuminous glands* (as the parotid in man and mammalia and the submaxillary in rabbits), partly *mucous glands* (as some of the small glands in the buccal cavity and the sublingual and submaxillary glands of many animals), and partly *mixed glands* (as the submaxillary gland in man). The alveoli of the albumin-glands contain cells which are rich in albumin, but contain no mucin. The alveoli of the mucin-glands contain cells rich in mucinogen or mucin but poor in albumin.

Cells rich in proteid also occur in the submaxillary and sublingual glands between the mucous cells and the membrana propria, which in a few cases takes the form of a crescent (lunula, according to GLANUZZI), and in other cases the cells rich in mucin are surrounded as by a ring, and sometimes certain alveoli may be completely filled. By continuous secretion the mucin-cells seem to give up all their mucin (EWALD, STÖHR), so that only albumin-cells are to be seen (HEIDENHAIN[1]). During rest the mucin is re-formed. According to the analyses of OIDTMANN[2] the salivary glands of a dog contain 790 p. m. water, 200 p. m. organic and 10 p. m. inorganic solids.

Among the solids we find *mucin*, *proteids*, amongst which *nucleoalbumin* or *nucleoproteid*, *nuclein*, *diastatic enzyme* and its *zymogen*,[3] besides *extractive bodies*, *leucin*, *xanthin bases*, and *mineral substances*.

The **saliva** is a mixture of the secretion of the above-mentioned groups of glands; therefore it is proper that we first study each of the different secretions by itself, and then the mixed saliva.

The **submaxillary saliva** in man may be easily collected by introducing a canula through the papillary opening into Wharton's duct.

The submaxillary saliva has not always the same composition or properties; this depends essentially upon the conditions under which the secretion takes place. That is to say, the secretion is partly dependent on the cerebral, partly on the sympathetic, nervous system. In consequence of this dependence the two distinct varieties of submaxillary secretion are distinguished as *chorda-* and *sympathetic* saliva. A third kind of saliva, the so-called *paralytic saliva*, is secreted after poisoning with curara or after the severing of the glandular nerves.

The difference between chorda- and sympathetic saliva (in dogs) consists chiefly in their quantitative constitution, namely, the less abundant sympathetic saliva is more viscous and richer in solids, especially in mucin, than the more abundant chorda-saliva. The specific gravity of the chorda-saliva of the dog is 1.0039–1.0056 and contains from 12–14 p. m. solids (ECKHARD[1]). The sympathetic has a specific gravity of 1.0075–1.018, with 16–28 p. m. solids. The gases of the chorda-saliva have been investigated by PFLÜGER.[2] He found 0.5–0.8% oxygen, 0.9–1% nitrogen, and 64.73–85.13% carbon dioxide—all results calculated at 0° C. and 760 mm. pressure. The greater part of the carbon dioxide was chemically combined.

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[1] In regard to these conditions see text-books on histology and the article "Die Absonderungsvorgänge" by Heidenhain in Hermann's Handbuch der Physiologie, Bd. 5, S. 57.

[2] Cit. from Gorup-Besanez, Lehrbuch d. physiol. Chem., 4. Aufl., S. 732. The figures there given amount to 1010 parts instead of 1000 parts.

[3] See especially Warren, Centralbl. f. Physiol., Bd. 8, S. 211.

The two kinds of submaxillary secretion just named have not thus far been separately studied in man. The secretion may be excited by a moral emotion, by mastication, and by irritating the mucous membrane of the mouth, especially with acid-tasting substances. The submaxillary saliva in man is ordinarily clear, rather thin, a little ropy, and froths easily. Its reaction is alkaline. The specific gravity is 1.002–1.003, and it contains 3.6–4.5 p. m. solids.[3] We find as organic constituents mucin, traces of proteid and diastatic enzyme, which is absent in several species of animals. The inorganic bodies are alkali chlorides, sodium and magnesium phosphates, besides bicarbonates of the alkalies and calcium. OEHL[4] finds 0.036 p. m. potassium sulphocyanide in this saliva.

The Sublingual Saliva.—The secretion of this saliva is also influenced by the cerebral and the sympathetic nervous system. The chorda-saliva, which is secreted only to a small extent, contains numerous salivary corpuscles, but is otherwise transparent and very ropy. Its reaction is alkaline and contains, according to HEIDENHAIN,[5] 27.5 p. m. solids (in dogs).

The sublingual secretion in man has been investigated by OEHL.[6] It was clear, mucilaginous, more alkaline than the submaxillary saliva, and contained mucin, diastatic enzyme, and potassium sulphocyanide.

Buccal mucus can only be obtained pure from animals by the method of BIDDER and SCHMIDT, which consists in tying the exit to all the large salivary glands and cutting off their secretion from the mouth. The quantity of liquid secreted under these circumstances (in dogs) was so very small that the investigators named were able to collect only 2 grms. buccal mucus in the course of twenty-four hours. It is a thick, ropy, sticky liquid containing mucin; it is rich in form-elements, above all in flat epithelium-cells, mucous cells, and salivary corpuscles. The quantity of solids in the buccal mucus of the dog is, according to BIDDER and SCHMIDT,[1] 9.98 p. m.

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[1] Cit. from Kühne, Lehrb. d. physiol. Chem., S. 7.

[2] Pflüger's Arch. Bd. 1.

[3] See Maly, Chemie der Verdauungssäfte und der Verdauung in Hermann's Handb., Bd. 5, Th. 2, S. 18.

[4] Canstatt's Jahresbericht d. Med., 1865, 1, S. 120.

[5] Studien d. physiol. Instituts zu Breslau, Heft 4.

[6] L. c.

Parotid Saliva. The secretion of this saliva is also partly dependent on the cerebral nervous system (n. glossopharyngeus) and partly on the sympathetic. The secretion may be excited by mental emotions and by irritation of the glandular nerves, either directly (in animals) or reflexly, by mechanical or chemical irritation of the mucous membrane of the mouth. Among the chemical irritants the acids take first place, while alkalies and pungent substances have little action. Sweet-tasting bodies, such as honey, are said to have no effect. Mastication has great influence in the secretion of parotid saliva, which is especially marked in certain herbivora.

Human parotid saliva may be collected by the introduction of a canula into STENSON'S duct. This saliva is thin, less alkaline than the submaxillary saliva (the first drops are sometimes neutral or acid), without special odor or taste. It contains a little albumin but no mucin, which is to be expected from the construction of the gland. It also contains a diastatic enzyme, which, however, is absent in many animals. The quantity of solids varies between 5 and 16 p. m. The specific gravity is 1.003–1.012. Potassium sulphocyanide seems to be present, though it is not a constant constituent. KÜLZ[2] found 1.46% oxygen, 3.2% nitrogen, and in all 66.7% carbon dioxide in human parotid saliva. The quantity of firmly combined carbon dioxide was 62%.

The mixed buccal saliva in man is a colorless, faintly opalescent, slightly ropy, easily frothing liquid without special odor or taste. It is made turbid by epithelium-cells, mucous and salivary corpuscles, and often by food residues. Like the submaxillary and parotid saliva, on exposure to the air it becomes covered with an incrustation consisting of calcium carbonate and a small quantity of an organic substance, or it gradually becomes cloudy. Its reaction is alkaline, but occasionally also acid. According to STICKER,[1] fresh saliva may be acid a few hours after a meal. Two or three hours after breakfast and four to five hours after dinner the maximum of acidity occurs, and it may also be faintly acid from midnight to morning. The specific gravity varies between 1.002 and 1.008, and the quantity of solids between 5 and 10 p. m. The solids, irrespective of the form-constituents mentioned, consist of *albumin*, *mucin*, *ptyalin*, and *mineral bodies*. It is also claimed that *urea* is a normal constituent of the saliva. The mineral bodies are alkali chlorides, bicarbonates of the alkalies and calcium, phosphates, and traces of sulphates and sulphocyanides.

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[1] Die Verdauungssäfte und der Stoffwechsel (Mitau and Leipzig, 1852), S. 5.

[2] Zeitschr. f. Biologie, Bd. 23.

Sulphocyanides, which, although not constant, occur in the saliva of man and certain animals, may be easily detected by first acidifying the saliva with hydrochloric acid and treating with a very dilute solution of ferric chloride. To make the test more conclusive it is best, as control, to take an equal quantity of acidified water and then add ferric chloride. Another, simpler method, proposed by GSCHEIDLEN,[2] consists in putting in a drop or two of the saliva on filter-paper which has previously been dipped in an amber-colored solution of ferric chloride containing hydrochloric acid, and then dried. Each drop of saliva containing sulphocyanide will give a reddish spot. If the quantity of sulphocyanide is so small that it cannot be detected directly, concentrate the saliva after the addition of a little alkali, acidify strongly with hydrochloric acid, and shake repeatedly with ether, evaporating the latter after the addition of water containing alkali over a gentle heat; then test the remaining liquid.

Ptyalin, or salivary diastase, is the amylolytic enzyme of the saliva. This enzyme is found in human saliva, but not in that of all animals. It occurs not only in adults, but also in new-born infants. ZWEIFEL[3] claims that the ptyalin in new-born infants occurs only in the parotid gland, but not in the submaxillary. In the latter it appears only two months after birth.

According to H. GOLDSCHMIDT[4] the saliva (parotid saliva) of the horse does not contain ptyalin, but a zymogen of the same, while in other animals and man the ptyalin is formed from the zymogen during secretion. In horses the zymogen is transformed into ptyalin during mastication, and the bacteria seem to give the impulse to this change. During precipitation with alcohol the zymogen is changed into ptyalin.

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[1] Deutsch. med. Zeitung, 1889. Cit. from Centralbl. f. Physiol., Bd. 3, S. 237.

[2] Maly's Jahresber., Bd. 4, S. 91.

[3] Untersuchungen über den Verdauungsapparat der Neugeborenen. Berlin, 1874.

[4] Zeitschr. f. physiol. Chem., Bd. 10.

Ptyalin has not been isolated in a pure form up to the present time. It can be obtained purest by COHNHEIM'S[1] method, which consists in carrying the enzyme down mechanically with a calcium-phosphate precipitate and washing the precipitate with water, which dissolves the ptyalin, and from which it can be obtained by precipitating with alcohol. For the study or demonstration of the action of ptyalin we may use a watery or glycerin extract of the salivary glands, or simply the saliva itself.

Ptyalin, like other enzymes, is characterized by its action. This consists in converting starch into dextrin and sugar. In regard to the process going on in this conversion we are not quite clear. In general it may be described as follows: In the first stages soluble starch or *amidulin* is formed. From this amidulin, erythrodextrin and sugar are produced by hydrolytic cleavage. The erythrodextrin then splits into α-achroodextrin and sugar. From this achroodextrin by splitting β-achroodextrin and sugar are formed, and finally this β-achroodextrin splits into sugar and γ-achroodextrin. According to a few investigators the number of dextrins formed, as intermediate steps is different. It is only within a very short time that we have been made clear as to the kind of sugar produced in this process. For a long time it was considered that dextrose was the sugar formed from starch and glycogen, but SEEGEN[2] and O. NASSE[3] have shown that this is not true.

MUSCULUS and v. MERING[4] have shown that the sugar formed by the action of saliva, amylopsin, and diastase upon starch and glycogen is in greatest part maltose. This has been substantiated by BROWN and HERON.[5] Lately E. KÜLZ and J. VOGEL[6] have demonstrated that in the saccharification of starch and glycogen isomaltose, maltose, and some dextrose are formed, the varying quantities depending upon the amount of ferment and length of time of digestion. As, according to TEBB,[1] the salivary glands, as well as the pancreas, contain an inverting enzyme, it is still undecided whether the formation of dextrose is due to the diastatic enzyme or to the invertin alone. According to RÖHMANN and HAMBURGER[2] the saliva contains diastase and glucase. The same is true for the pancreatic juice and intestinal juice. In relation to the blood-serum all these secretions are relatively poor in glucase, and this is especially true for saliva.

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[1] Virchow's Arch., Bd. 28.

[2] Centralbl. f. d. med. Wissensch., 1876, S. 851, and Pflüger's Arch., Bd. 19.

[3] Pflüger's Arch., Bd. 14.

[4] Zeitschr. f. physiol Chem., Bd. 2.

[5] Liebig's Annalen, Bdd. 199 and 204.

[6] Zeitschr. f. Biologie, Bd. 31.

Ptyalin is not identical with malt diastase. It is most active at about + 40° C., while, according to CHITTENDEN and MARTIN,[3] LINTNER, and ECKHARD,[4] malt diastase is most active at + 50° to 55° C.

The action of ptyalin in various *reactions* has been the subject of numerous investigations.[5] Naturally the alkaline saliva is very active, but it is not as active as when neutral. It may be still more active under circumstances in faintly acid reaction, and according to CHITTENDEN and SMITH it acts better when enough hydrochloric acid is added to saturate the proteids present than when only simply neutralized. When the acid combined proteid exceeds a certain amount, then the diastatic action is diminished. The addition of alkali to the saliva decreases its diastatic action; on neutralizing the alkali with acid or carbon dioxide the retarding or preventive action of the alkali is arrested. According to SCHIERBECK carbon dioxide has an accelerating action in neutral liquids, while EBSTEIN claims that it has as a rule a retarding action. Organic as well as inorganic acids, when added in sufficient quantity, may stop the diastatic action entirely. The degree of acidity necessary in this case is not always the same for a certain acid, but is dependent upon the quantity of ferment. The same degree of acidity in the presence of large amounts of ferment has a weaker action than in the presence of smaller quantities. Hydrochloric acid is of special physiological interest in this regard, namely, it prevents the formation of sugar even in very small amounts (0.03 p. m.). Hydrochloric acid has not only the property of preventing the formation of sugar, but, as shown by LANGLEY, NYLÉN, and others, may entirely destroy the enzyme. This is important in regard to the physiological significance of the saliva. That boiled starch (paste) is quickly, and unboiled starch only slowly, converted into sugar is also of interest. Various kinds of unboiled starch are converted with different degrees of rapidity.

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[1] Journal of Physiol., Vol. 15.

[2] Ber. d. deutsch. chem. Gesellsch., Bd. 27, and Pflüger's Arch., Bd. 60.

[3] Studies from the Laborat. of Physiol. Chem. of Yale College, Vol. 1, 1885.

[4] Journ. f. prakt. Chem., N. F. Bd. 41.

[5] See Hammarsten, Maly's Jahresber., Bd. 1; Chittenden and Griswold, *ibid.*, Bd. 11; Langley, Journal of Physiol., Vol. 3; Nylén, Maly's Jahresber., Bd. 12, S. 241; Chittenden and Ely, *ibid.*, S. 242; Langley and Eves, Journal of Physiol., Vol. 4; Chittenden and Smith, Yale College Studies, Vol. 1, 1885, p. 1; John, Centralbl. f. klin. Med., Bd. 12; Schlesinger, Virchow's Arch., Bd. 125; Shierbeck, Skand. Arch. f. Physiol., Bd. 3; Ebstein and C. Schulze, Virchow's Arch., Bd. 134.

The *rapidity* with which ptyalin acts increases, at least under conditions otherwise favorable, with the *amount of enzyme* and with an increasing *temperature* to a little above + 40° C. *Foreign substances*, such as metallic salts,[1] have different effects. Certain salts even in small quantities completely arrest the action; for example, $HgCl_2$ accomplishes this result by the presence of only 0.05 p. m. Other salts, such as magnesium sulphate, in small quantities (0.25 p. m.) accelerate, and in larger quantities (5 p. m.) check the action. The presence of peptone has an accelerating action on the sugar formation (CHITTENDEN and SMITH and others). The accumulation of the products of the amylolytic decomposition also checks the action of the saliva. This has been shown by special experiments made by SH. LEA.[2] He made parallel experiments with digestions in test-tubes and in dialyzers, and found on the removal of the products of the amylolytic decomposition by dialysis that the formation of sugar took place quicker, but also that considerably more maltose and less dextrin was formed.

To show the action of saliva or ptyalin on starch the three ordinary tests for dextrose may be used, namely, MOORE'S or TROMMER'S test or the *bismuth test* (see Chapter XV). It is also necessary, as a control, to first test the starch-paste and the saliva for the presence of dextrose. The steps formed in the transformation of starch into amidulin, erythrodextrin, and achroodextrin may be shown by testing with iodine.

The *quantitative composition* of the mixed saliva must vary considerably, not only because of individual differences, but also because under varying conditions there may be an unequal division of the secretion from the different glands. We give below a few analyses of human saliva as example of its composition. The results are in parts per 1000.

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[1] See O. Nasse, Pflüger's Arch., Bd. 11, and Chittenden and Painter, Yale College Studies, Vol. 1, 1885, p. 52.

[2] Journ. of Physiol., Bd. 11.

	BERZELIUS.	JACUBOWITSCH.	FRERICHS.	TIEDEMANN and GMELIN.	HERTER.	LEHMANN.	HAMMERBACHER.[1]
Water	992.9	995.16	994.1	988.3	994.7		994.2
Solids	7.1	4.84	5.9	11.7	5.3	3.5–8.4 in filtered saliva.	5.8
Mucus and epithelium	1.4	1.62	2.13				2.2
Soluble organic substances (Ptyalin of early investigators).	3.8	1.34	1.42		3.27		1.4
Sulphocyanides		0.06	0.10			0.064 to 0.09	0.04
Salts	1.9	1.82	2.19		1.03		2.2

HAMMERBACHER found in 1000 parts of the ash from human saliva: potash 457.2, soda 95.9, iron oxide 50.11, magnesia 1.55, sulphuric anhydride (SO_3) 63.8, phosphoric anhydride (P_2O_5) 188.48, and chlorine 183.52.

The quantity of saliva secreted during 24 hours cannot be exactly determined, but has been calculated by BIDDER and SCHMIDT[2] to be 1400–1500 grms. The most abundant secretion occurs during meal-times. According to the calculations and determinations of TUCZEK[3] in man, 1 grm. of gland yields 13 grms. secretion in the course of one hour during mastication. These figures correspond fairly well with those representing the average secretion from 1 grm. of gland in animals, namely, 14.2 grms. in the horse and 8 grms. in oxen. The quantity of secretion pèr hour may be 8 to 14 times greater than the entire mass of glands, and there is probably no gland in the entire body, as far as we know at present—the kidneys not excepted—whose ability of secretion under physiological conditions equals that of the salivary glands. A remarkably abundant secretion of saliva is induced by pilocarpin, while atropin, on the contrary, prevents it.

Though an abundant secretion of saliva is produced, as a rule, by an increased supply of blood, still it is not a simple filtration process, as seen from the following circumstances. The secretion-pressure is greater than the blood-pressure in the carotid, and in poisoning by atropin, which paralyzes the secretory nerves, an increased supply of blood is produced by irritation of the chorda, but no secretion. The salivary glands have moreover a specific property of eliminating certain substances, such as potassium salts (SALKOWSKI),[1] iodine, and bromine combinations, but not others, such as iron combinations. It is also noticeable that the saliva is richer in solids when it is eliminated quickly by gradually increased irritation, and in larger quantities than when the secretion is slower and less abundant (HEIDENHAIN).[2] The amount of salts increases also to a certain degree by an increasing rapidity of elimination (HEIDENHAIN, WERTHER,[3] LANGLEY and FLETCHER,[4] NOVI[5]).

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[1] Zeitschr. f. physiol. Chem., Bd. 5. The other analyses are cited from Maly, Chemie der Verdauungssäfte, Hermann's Handbuch d. Physiol., Bd. 5, Th. 2, S. 14.

[2] L. c., S. 13.

[3] Zeitschr. f. Biologie, Bd. 12.

The chemical changes taking place during secretion are unknown, but it is probable that, like the secretion processes in general, the secretion of saliva is closely connected with the processes in the cells. The chemical processes going on in these cells during secretion are still unknown. HEIDENHAIN claims that the mucin cells of the submaxillary gland are destroyed during secretion, and in the period of rest the mucin or mucinogen reappears in these cells. EWALD[6] claims that they only discharge their mucin. These observations still do not throw any light upon the chemical processes going on.

The Physiological Importance of the Saliva. The quantity of water in the saliva renders possible the effects of certain bodies on the organs of taste, and it also serves as a solvent for a part of the nutritive substances. The importance of the saliva in mastication is especially marked in herbivora, and there is no question of its importance in facilitating the act of swallowing. The power of converting starch into sugar does not belong to the saliva of all animals, and even when it possesses this property the intensity varies in different animals. In man, whose saliva forms sugar rapidly, a formation of sugar from (boiled) starch undoubtedly takes place in the mouth, but how far this action goes on after the morsel has entered the stomach depends upon the rapidity with which the acid gastric juice mixes with the swallowed food, and also upon the relative amounts of the gastric juice and food in the stomach. The large quantity of water which is swallowed with the saliva must be absorbed and pass into the blood, and it must go through an intermediate circulation in the organism. Thus the organism possesses in the saliva an active medium by which a constant stream, conveying the dissolved and finely divided bodies, passes into the blood from the intestinal canal during digestion.

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[1] Virchow's Arch., Bd. 53.

[2] Pflüger's Arch., Bd. 17.

[3] *Ibid.*, Bd. 38.

[4] Proc. Roy. Soc., Vol. 45, and especially Philos. Trans., Vol. 180.

[5] Du Bois-Reymond's Arch., 1888.

[6] See Heidenhain in Hermann's Handb., Bd. 5, Th. 1, S. 64, etc.

Salivary Concrements. The so-called tartar is yellow, gray, yellowish gray, brown or black, and has a stratified structure. It may contain more than 200 p. m. organic substances, which consist of mucin, epithelium, and LEPTOTHRIX-CHAINS. The chief part of the inorganic constituents consists of calcium carbonate and phosphate. The salivary calculi may vary in size from that of a small grain to that of a pea or still larger (a salivary calculus has been found weighing 18.6 grms.), and it contains a variable quantity of organic substances, 50–380 p. m., which remain on extracting the calculus with hydrochloric acid. The chief inorganic constituent is calcium carbonate.

II. The Glands of the Mucous Membrane of the Stomach, and the Gastric Juice.

Since of old, the glands of the mucous coat of the stomach have been divided into two distinct kinds. Those which occur in the greatest abundance and which have the greatest size in the fundus are called *fundus glands*, also rennin or pepsin glands. Those which occur only in the neighborhood of the pylorus have received the name of *pyloric glands*, sometimes also, though incorrectly, called *mucous glands*. The mucous coating of the stomach is covered throughout with a layer of columnar epithelium which is generally considered as consisting of goblet cells that produce mucus by a metamorphosis of the protoplasm.

The **fundus glands** contain two kinds of cells: ADELOMORPHIC or chief cells, and DELOMORPHIC or PARIETAL cells, the latter formerly called RENNIN or pepsin cells. Both kinds consist of protoplasm rich in proteids; but their relationship to coloring matters seems to show that the albuminous bodies of both are not identical. The nucleus must consist chiefly of nuclein. Besides the above-mentioned constituents the fundus glands contain as more specific constituents two *zymogens*, which are the mother-substances of the *pepsin* and the *rennin*, besides a small quantity of fat and cholesterin.

The pyloric glands contain cells which are generally considered as related to the above-mentioned chief cells of the fundus glands. As these glands were formerly thought to contain a larger quantity of mucin, they were also called mucous glands. According to HEIDENHAIN, independent of the columnar epithelium of the excretory ducts, they take no part worthy of mention in the formation of mucus, which, according to his views, is effected by the epithelium covering the mucous membrane. The pyloric glands also seem to contain the *zymogens* referred to above. Alkali chlorides, alkali phosphates, and calcium phosphates are found in the mucous coating of the stomach.

LIEBERMANN[1] has obtained an acid-reacting residue on digesting the mucosa of the stomach with pepsin hydrochloric acid, which strangely contained no nuclein, but only a proteid containing lecithin, called lecithalbumin. To this lecithalbumin he ascribes a great importance in the secretion of hydrochloric acid (see below).

The Gastric Juice. The observations of HELM[2] and BEAUMONT[3] on persons with gastric fistula led to the suggestion that gastric fistulas be made on animals, and this operation was first performed by BASSOW[4] in 1842 on a dog. VERNEUIL[5] performed the same on a man in 1876 with successful results. These fistulas in animals afford an excellent means of studying the secretion of gastric juice and also the stomachic digestion.

In a fasting condition the mucous coat is often nearly dry; sometimes, especially in certain herbivora, it is covered with a layer of viscid so-called mucus. If food is introduced into the stomach, or if the mucous membrane is irritated in some way, then a secretion of a thin, acid fluid, the real gastric juice, takes place. The secretion may be produced by mechanical or thermal irritation (introduction of cold water or pieces of ice into the stomach), or by chemical irritants. Among the latter we include alcohol and ether, which when in too great concentration do not produce a physiological secretion, but a transudation of a neutral or faintly alkaline fluid containing albumin. To this class of irritants belong carbon dioxide and hydrochloric acid; the last especially increases the secretion of pepsin (Jaworsky[1]), spices, meat extracts, neutral salts, such as NaCl (which acts like alcohol in too great concentration), and alkali carbonates. The alkali carbonates are supposed by certain investigators to first neutralize the acid and then produce a continuous secretion of acid gastric juice. The statements in regard to the action of different bodies on the secretion of gastric juice are still rather uncertain and often contradictory.

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[1] Pflüger's Arch., Bd. 50.

[2] Helm, Zwei Krankengeschichten. Wien, 1803. Cit. from Hermann's Handbuch, Bd. 5, Th. 2, S. 39.

[3] "The Physiology of Digestion," 1833.

[4] Bull. de la soc. des natur. de Moscou, Tome 16. Cited from Maly in Hermann's Handbuch, Bd. 5, S. 38.

[5] See Ch. Richet, Du suc gastrique chez l'homme et les animaux. Paris, 1878, p. 158.

The secretion of gastric juice is reflexly stimulated from the mouth. After the introduction of water into the stomach a relatively scanty and not less constant flow of secretion takes place; while on the contrary if digestible food is introduced a more abundant and continuous secretion is observed (Schiff,[2] Heidenhain[3]). But in these cases the secretion does not take place immediately, but only after the absorption of the soluble bodies has commenced. This fact justifies the usual custom of commencing a meal with fluid nutritives, such as soup. The beautiful experiments made by Pawlow and Schoumow-Simanowsky[4] have shown that the secretion of gastric juice is stimulated reflexly from the mouth, and also that this reflex is discontinued on cutting through the vagi, and that the secretion in the stomachic glands is caused by the central nervous system through special secretory nerve-fibres, analogous to the secretion of saliva and pancreatic juice.

The Qualitative and Quantitative Composition of the Gastric Juice. The gastric juice, which can hardly be obtained pure and free from residues of the food or from mucus and saliva, is a clear, or only very faintly cloudy, and in man nearly colorless fluid of an insipid, acid taste and strong acid reaction. It contains, as form-elements, *glandular cells* or their *nuclei*, *mucus-corpuscles*, and more or less changed *columnar epithelium.*

The acid reaction of the gastric juice depends on the presence of free acid, which, as we have learned from the investigations of C. Schmidt,[5] Richet,[6] and others, consists, when the gastric juice is pure and free from particles of food, chiefly or nearly so of *hydro-*

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[1] Deutsch. med. Wochenschr., 1887.

[2] Leçons sur la physiol. de la digestion, Tome 2, 1867.

[3] Pflüger's Arch., Bd. 19.

[4] Du Bois-Reymond's Arch., 1895.

[5] Bidder and Schmidt, Die Verdauungssäfte, etc., S. 44.

[6] L. c.

chloric acid. Contejean[1] has regularly found traces of lactic acid in the pure gastric juice of fasting dogs. After partaking of food, especially after a meal rich in carbohydrates, lactic acid occurs abundantly, and sometimes acetic and butyric acids. The quantity of free hydrochloric acid in the gastric juice of sheep is about 1.2 p. m., and in dogs, according to the ordinary statements, about 2–3 p. m. Schoumow-Simanowsky[2] has observed a considerably higher degree of acidity in perfectly pure and fresh gastric juice of a dog, namely, 4.6–5.8 p. m. Riasantsew[3] states that the gastric juice of the cat is very similar to that of the dog and has about the same degree of acidity, 4.11–5.84 p. m., and an average of 5.20 p. m. Richet[4] found as average for 80 determinations of human gastric juice 1.7 p. m. free hydrochloric acid, with a variation between 0.5 and 3 p. m. According to Szabo,[5] Ewald,[6] and others, the human gastric juice contains usually about 2–3 p. m. HCl. Richet has shown that the acid gastric juice acts in many respects different from free hydrochloric acid of the same concentration, and he concludes from this that the hydrochloric acid is not free, but combined with organic substances (leucin). Contejean is of the same opinion, and has found that gastric juice dissolves cobalt hydrocarbonate with more difficulty and slower than a hydrochloric acid of the same concentration.

Perfectly fresh gastric juice seems to contain a little coagulable proteid, but contains *peptone* and *albumoses* on standing for some time. Among the organic bodies a little *mucin* is found and two enzymes, *pepsin* and *rennin*, especially in man. The sulphocyanic acid found by Kelling[7] in the contents of the stomach is considered by Nencki and Schoumow-Simanowsky[8] as a normal constituent of pure saliva-free gastric juice of dogs.

The specific gravity of gastric juice is low, 1.001–1.010. It is therefore correspondingly poor in solids. As examples of the composition of different kinds of gastric juice the analyses of C. SCHMIDT[1] are here given. It must be remarked that the human gastric juice analyzed was diluted by saliva and water and should therefore not be considered as normal. The figures are parts per 1000.

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[1] Contrib. à l'étude de la physiol. de l'estomac. Thesis. Paris, 1892. Maly's Jahresber., Bd 22, S. 293.

[2] Arch. f. exp. Path. u. Pharm., Bd. 33.

[3] Arch. des Sciences biol. de St. Pétersbourg, Tome 3.

[4] L. c.

[5] Zeitschr. f. physiol. Chem., Bd. 1.

[6] C. A. Ewald, Klinik der Verdauungskrankheiten, 1890.

[7] Zeitschr. f. physiol Chem., Bd. 18.

[8] Arch. f. exp. Path. u. Pharm., Bd. 34, and Ber. d. deutsch. Chem. Gesellsch., Bd. 28.

	Human Gastric Juice mixed with Saliva.	Gastric Juice from Dog free from Saliva.	Gastric Juice from Dog containing Saliva.	Gastric Juice of Sheep.
Water	994.40	973.0	971.2	986.15
Solids	5.60	27.0	28.8	13.85
Organic substance	3.19	17.1	17.3	4.05
NaCl	1.46	2.5	3.1	4.36
$CaCl_2$	0.06	0.6	1.7	0.11
KCl	0.55	1.1	1.1	1.52
NH_4Cl	....	0.5	0.5	0.47
Free hydrochloric acid (HCl)	0.20	3.1	2.3	1.23
$Ca_3(PO_4)_2$	0.12	1.7	2.3	1.18
$Mg_3(PO_4)_2$		0.2	0.3	0.57
$FePO_4$		0.1	0.1	0.33

The other physiologically important constituents of gastric juice are *pepsin* and *rennin*.

Pepsin. This enzyme is found, with the exception of certain fishes, in all vertebrates thus far investigated.

Pepsin occurs in adults and in new-born infants. This condition is different in new-born animals. While in a few herbivora, such as the rabbit, pepsin occurs in the mucous coat before birth, this enzyme is entirely absent at the birth of those carnivora which have thus far been examined, such as the dog and cat.

In various invertebrates a ferment has also been found which has a proteolytic action in acid solutions. It has been shown that this enzyme, nevertheless, is not in all animals identical with ordinary pepsin. DARWIN has further found that certain plants which feed upon insects secrete an acid juice which dissolves proteid, but it is still doubtful whether these plants contain any pepsin. v. GORUP-BESANEZ[2] has isolated from vetch-seed an enzyme which acts like pepsin, but whose identity with pepsin is doubtful.

Pepsin is as difficult to isolate in a pure condition as other enzymes.[3] The purest pepsin was that prepared by BRÜCKE and

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[1] Cit. from v. Gorup-Besanez, Lehrbuch d. physiol. Chem., 4. Aufl., S. 494.

[2] Ber. d. deutsch. chem. Gesellsch., Bdd. 7 and 8.

[3] Schoumow-Simanowsky, Arch. f. exp. Path. u. Pharm., Bd. 33, has observed that the pure, fresh gastric juice of a dog deposits a protein substance containing chlorine, on cooling, and this he considers as pure pepsin. This substance is, however, precipitated by certain proteid reagents which even do

SUNDBERG; this gave negative results with most reagents for proteids. Pepsin, therefore, does not seem to be a true albuminous substance. It is, at least in the impure condition, soluble in water and glycerin. It is precipitated by alcohol, but only slowly destroyed. It is quickly destroyed by heating its watery solution to boiling. According to BIERNACKI[1] pepsin in neutral solutions is destroyed by heating to + 55° C. In the presence of 2 p. m. HCl a temperature of 55° C. is without action; the pepsin is destroyed by heating to 65° C. for five minutes. On adding peptone and certain salts the pepsin may be heated to 70° C. without decomposing. In the dry state it can, on the contrary, be heated to over 100° C. without losing its physiological action. The only property which is characteristic of pepsin is that it dissolves proteid bodies in acid, but not in neutral or alkaline, solutions with the formation of albumoses and peptones.

The methods for the preparation of relatively pure pepsin depend, as a rule, upon its property of being thrown down with finely divided precipitates of other bodies, such as calcium phosphate or cholesterin. The rather complicated methods of BRÜCKE[2] and SUNDBERG[3] are based upon this property. A relatively pure pepsin solution intended for digestion tests and of effective action may be prepared by the following method as suggested by MALY.[4] The mucous membrane (of the pig's stomach) is treated with water containing phosphoric acid, and the filtrate precipitated by lime-water; the precipitate, which contains the pepsin, is then dissolved in water by the addition of hydrochloric acid, and the salts removed by dialysis, by which means the pepsin which does not diffuse remains in the dialyzer. A pepsin solution somewhat impure but rich in pepsin, and which can be kept for years, may be obtained if, as suggested by v. WITTICHS,[5] we extract the finely divided mucous membrane with glycerin, or better with glycerin which contains 1 p. m. HCl. To each part by weight of the mucous coat add 10–20 parts glycerin. This is filtered after 8–14 days. The pepsin (together with much albumin) may be precipitated by alcohol from this extract. If this extract is to be used directly for digestion tests, then to 100 c. c. of water which has been acidified with 1–4 p. m. HCl add 2–3 c. c. of the extract.

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not precipitate very powerful commercial pepsin, and therefore it cannot be a pure enzyme.

[1] Zeitschr. f. Biologie, Bd. 28.

[2] Wien. Sitzungsber., Bd. 43.

[3] Zeitschr. f. physiol. Chem., Bd. 9.

[4] Pflüger's Arch., Bd. 9.

[5] *Ibid.*, Bd. 2.

For digestion tests an infusion of the mucous membrane of the stomach may be used directly in many cases. The mucous coat is carefully washed with water (if a pig's stomach is used) and finely cut; if a calf's stomach is employed, only the outer layer of the mucous coat is scraped off with a watch-glass or the back of a knife. The pieces of mucous membrane or the slimy masses obtained by scraping are rubbed with pure quartz-sand, treated with acidified water, and allowed to stand for 24 hours in a cool place and then filtered.

In the preparation of artificial gastric juice that part only of the mucous coat richest in pepsin is used; the pyloric part is of little value. A strong, impure infusion may generally be obtained from the pig's stomach, while a relatively pure and powerful infusion is obtained from the stomach of birds (hens). The stomachs of fish (pike) also yield a tolerably pure and active infusion. An active and rather pure artificial gastric juice may be prepared by scraping the inner layers of a calf's stomach from which the pyloric end has been removed. For a medium-sized calf's stomach 1000 c. c. of acidified water must be used.

The degree of acidity required in the infusion depends upon the use to which the gastric juice is to be put. If it is to be employed in the digestion of fibrin, an acidity of 1 p. m. HCl must be selected, while, on the contrary, if it is to be used for the digestion of hard-boiled-egg albumin, an acidity of 2–3 p. m. HCl is preferable. This last-mentioned degree of acidity is generally the better, because the infusion is preserved thereby, and at all events it is so rich in pepsin that it may be diluted with water until it has an acidity of 1 p. m. HCl without losing any of its solvent action on unboiled fibrin.

The preparation of acid infusions is nowadays unnecessary on account of the ability of getting various pepsin preparations in commerce which have a remarkable activity. Such a pepsin preparation can be purified when necessary by following the method suggested by KÜHNE.[1] Precipitate the pepsin together with the albumoses by ammonium sulphate, press the precipitate and dissolve in dilute hydrochloric acid, and let it undergo auto-digestion. On repeating this again and then removing the salts by dialysis we obtain an extraordinarily active pepsin, but which is still less pure than when obtained by the methods of BRÜCKE and SUNDBERG.

The Action of Pepsin on Proteids. Pepsin is inactive in neutral or alkaline reactions, but in acid liquids it dissolves coagulated albuminous bodies. The proteid always swells and becomes transparent before it dissolves. Unboiled fibrin swells up in a solution containing 1 p. m. HCl, forming a gelatinous mass, and does not dissolve at ordinary temperature within a couple of days. Upon the addition of a little pepsin, however, this swollen mass dissolves quickly at an ordinary temperature. Hard-boiled-egg albumin, cut in thin pieces with sharp edges, is not perceptibly changed by dilute acid (2–4 p. m. HCl) at the temperature of the body in the course of several hours. But the simultaneous presence of pepsin causes the edges to become clear and transparent, blunt and swollen, and the albumin gradually dissolves.

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[1] Zeitschr. f. Biologie, Bd. 22, S. 428.

From what has been said above in regard to pepsin, it follows that proteids may be employed as a means of detecting pepsin in liquids. Fibrin may be employed as well as hard-boiled-egg albumin, which latter is used in the form of slices with sharp edges. As the fibrin is easily digested at the normal temperature, while the pepsin test with egg-albumin requires the temperature of the body, and as the test with fibrin is somewhat more delicate, it is often preferred to that with egg-albumin. When we speak of the "*pepsin test*" without further explanation, we ordinarily understand it as the test with fibrin.

This test nevertheless requires care. The fibrin used should be ox fibrin and not pig fibrin, which last is dissolved too readily with dilute acid alone. The unboiled fibrin may be dissolved by acid alone without pepsin, but this generally requires more time. In testing with unboiled fibrin at normal temperature, it is advisable to make a control test with another portion of the same fibrin with acid alone. Since at the temperature of the body unboiled fibrin is easier dissolved by acid alone, it is best always to work with boiled fibrin.

As pepsin has not, thus far, been prepared in a positively pure condition, it is impossible to determine the absolute quantity of pepsin in a liquid. It is only possible to compare the relative amounts of pepsin in two or more liquids, which may be done in several ways. As the best of these we give the following method as suggest by BRÜCKE.

If two pepsin solutions *A* and *B* are to be compared with each other relatively to the amounts of pepsin they contain, they must first be brought to the proper degree of acidity, about 1 p. m. HCl, care being taken that one is not more diluted than the other. Then prepare a large number of specimens of each solution by diluting with hydrochloric acid of 1 p. m. HCl, so that they contain respectively $\frac{1}{2}$, $\frac{1}{4}$. $\frac{1}{8}$. $\frac{1}{16}$, $\frac{1}{32}$, and so on, the amount of pepsin in the original liquid being 1. If the original quantity of pepsin in the two liquids is designated by p and p', we then have the two series of liquids:

A	B
$1p$	$1p'$
$\frac{1}{2}p$	$\frac{1}{2}p'$
$\frac{1}{4}p$	$\frac{1}{4}p'$
$\frac{1}{8}p$	$\frac{1}{8}p'$
$\frac{1}{16}p$	$\frac{1}{16}p'$
$\frac{1}{32}p$	$\frac{1}{32}p'$

Then a small piece of boiled-egg albumin, obtained by cutting thin slices with a cork-cutter, is placed in each test, or a small flake of fibrin is added. Of course care must be taken to add the same-sized slice of egg-albumin or flake of fibrin. Now observe and note exactly the time when each test of the two series begins to digest and when it ends, and it will be found that certain tests of one series make about the same progress as certain tests of the other series. It may be inferred from this that they contain about the same quantity of pepsin. As example, it is found in one series of tests that the digestive rapidity of the tests $p\,\frac{1}{8}$, $p\,\frac{1}{16}$, $p\,\frac{1}{32}$ is about the same as the tests $p'\,\frac{1}{2}$, $p'\,\frac{1}{4}$, $p'\,\frac{1}{8}$; therefore we conclude that the liquid *A* is about four times as rich in pepsin as the liquid *B*.

Another method as suggested by METTE[1] gives more exact results according to the investigations of SAMOJLOFF.[2] Draw up liquid white of egg in a glass tube of about 1 to 2 mm. diameter and coagulate the albumin in the tube by heating, cut the ends of the tube off sharply, add two tubes to each test-tube with a few cc. of acid pepsin solution, allow to digest at the bodily temperature, and after a certain time measure the lineal extent of the digested layer of albumin in the various tests. From the rapidity of digestion expressed from the extent of the digested layer, we can calculate the relative quantity of pepsin, according to the rule first found by SCHÜTZ,[3] that the energy of digestion of different pepsin solutions is the square root of the quantity of pepsin it contains. This rule, however, only applies to sufficiently dilute pepsin solutions.

The *rapidity of the pepsin digestion* depends on several circumstances. Thus *different acids* are unequal in their action; hydrochloric acid shows a more powerful action than any other, whether an organic or an inorganic acid. The *degree of acidity* is also of the greatest importance. With hydrochloric acid the degree of acidity is not the same for different proteid bodies. For fibrin it is 0.8–1 p. m., for myosin, casein, and vegetable albumin about 1 p. m., for hard-boiled-egg albumin, on the contrary, about 2.5 p. m. The rapidity of the digestion increases, at least to a certain point, with the *quantity of pepsin* present, unless the pepsin added is contaminated by a large quantity of products of digestion, which may prevent its action. The *accumulation of products of digestion* has a retarding action on digestion, although, according to CHITTENDEN and AMERMAN,[4] the removal of the digestion products by means of dialysis does not essentially change the relationship between the albumoses and true peptones. Pepsin acts slower at low *temperatures* than it does at higher. It is even active in the neighborhood of 0°C., but digestion takes place very slowly at this temperature. With increasing temperature the rapidity of digestion also increases until about 40° C., when the maximum is reached.

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[1] Cited from Samojloff. See foot-note 2.

[2] Arch. des Sciences biol. de St. Pétersbourg, Tome 2, S. 699.

[3] Zeitschr. f. physiol. Chem., Bd. 9, S. 577.

[4] Journal of Physiol., 1893.

According to the investigations of FLAUM[1] it is probable that the relationship between albumoses and peptones remains the same, irrespective of whether the digestion took place at a low or high temperature as long as the digestion is continuous for some time. If the *swelling up of the proteid* is prevented, as by the addition of neutral salts, such as NaCl in sufficient amounts, or by the addition of bile to the acid liquid, digestion can be prevented to a greater or less extent. *Foreign bodies* of different kinds produce different actions, in which naturally the variable quantities in which they are added are of the greatest importance. Salicylic acid and carbolic acid hinder the digestion, while arsenious acid promotes it (CHITTENDEN), and hydrocyanic acid is relatively indifferent. Alcohol in large quantities (10% and above) disturbs the digestion, while small quantities act indifferently. Metallic salts in very small quantities may indeed sometimes accelerate digestion, but otherwise they tend to retard it. The action of metallic salts in different cases can be explained in different ways, but they often seem to form with proteids insoluble or difficultly-soluble combinations. The alkaloids may also retard the pepsin digestion (CHITTENDEN and ALLEN).[2] A very large number of observations have been made in regard to the action of foreign substances on artificial pepsin digestion, but as these observations have not given any direct result in regard to the action of these same substances on natural digestion, we will not here further discuss them.

The Products of the Digestion of Proteids by Means of Pepsin and Acid. In the digestion of nucleoproteids or nucleo-albumins an insoluble residue of nuclein or pseudo-nuclein always remains. With experiments on casesin SALKOWSKI[3] has shown that the paranuclein first split off which contains according to WILLDENOW,[4] phosphorus in organic combination, may be dissolved by continuous digestion. Some orthophosphoric acid is hereby split off, but an organic phosphorized acid is also formed.

Fibrin also yields an insoluble residue, which consists, at least in great part, of nuclein, derived from the form-elements enclosed in the blood-clot. This residue which remains in the digestion of certain albuminous bodies is called *dyspeptone* by MEISSNER.[1] If the solution is filtered after a finished digestion and neutralized, it gives in different cases a more or less abundant precipitate of acid albuminate, or a mixture of albuminates called *parapeptone* by MEISSNER. After filtering this precipitate and concentrating the filtrate again, some proteid often separates in the warmth. If this precipitate be filtered, the filtrate now contains *albumoses* and *peptones* in the ordinary sense, while the so-called true peptone of KÜHNE may sometimes be entirely absent, and in general is obtained in quantity worth mentioning only after a more continuous and intensive digestion. The relationship between the albumoses and peptones in the ordinary sense changes very much in different cases and in the digestion of various albuminous bodies. For instance, a larger quantity of primary albumoses is obtained from fibrin than from hard-boiled egg albumin or from the proteids of meat. In the digestion of unboiled fibrin an intermediate product may be obtained in the earlier stages of the digestion—a globulin which coagulates at + 55° C. (HASEBROEK[2]). For information in regard to the different albumoses and peptones which are formed in pepsin digestion, the reader is referred to previous pages (33–39).

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[1] Zeitschr. f. Biologie, Bd. 28.

[2] Yale College Studies, Vol. 1, p. 76. See also Chittenden and Stewart, *ibid.*, Vol. 3, p. 60.

[3] Centralbl. f. d. med. Wissensch., 1893, S. 385 and 467. See also Sontag, *ibid.*, S. 419, and Moraczewski, Zeitschr. f. physiol. Chem., Bd. 20.

[4] "Zur Kenntniss der peptischen Verdauung des Kaseins." Inaug. Diss. Bern., 1893.

Action of Pepsin Hydrochloric Acid on other Bodies. The *gelatin-forming substance* of the connective tissue, of the cartilage and of the bones, from which last the acid only dissolves the inorganic substances, is converted into *gelatin* by digesting with gastric juice. The gelatin is further changed so that it loses its property of gelatinizing and is converted into a so-called gelatin peptone (see page 55). True *mucin* (from the submaxillary) is dissolved by the gastric juice and yields a substance similar to peptone and a reducing substance similar to that obtained by boiling with a mineral acid. *Elastin* is dissolved more slowly and yields the above-mentioned substances (page 52). *Keratin* and the epidermis formation are insoluble. *Nuclein* is not dissolved and the cell-nuclei are therefore insoluble in gastric juice. The *animal cell-membrane* is, as a rule, more easily dissolved the nearer it stands to elastin, and it dissolves with greater difficulty the more closely it is related to keratin. The *membrane of the plant-cell* is not dissolved. *Oxyhæmogloblin* is changed into hæmatin and acid albuminate, the latter undergoing further digestion. It is for this reason that blood is changed into a dark-brown mass in the stomach. The gastric juice does not act on *fat*, but, on the contrary, on fatty tissue, dissolving the cell-membrane, setting the fat free. Gastric juice has no action on starch or the simple varieties of sugar. The statements in regard to the ability of gastric juice to invert cane-sugar are very contradictory. At least, this action of the gastric juice is not constant, and, according to VOIT,[1] if it is present at all, it is probably due to the action of the acid.

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[1] The works of Meissner on pepsin digestion are found in Zeitschr. f. rat. Med., Bdd. 7, 8, 10, 12, and 14.

[2] Zeitschr. f. physiol. Chem., Bd. 11.

Pepsin alone, as above stated, has no action on proteids, and an acid of the intensity of the gastric juice can only very slowly, if at all, dissolve coagulated albumin at the temperature of the body. Pepsin and acid together not only act more quickly, but qualitatively they act otherwise than the acid alone. If liquid proteid is digested with hydrochloric acid of 2 p. m., it is converted into acid albuminates; but if pepsin is previously added to the acid, the formation of syntonin takes place essentially slower under the same conditions (MEISSNER). From this it is inferred that a part of the hydrochloric acid is combined with the pepsin, and we have here a proof of the existence of a paired acid, called by C. SCHMIDT *pepsin hydrochloric acid.*

It has been further suggested that this hypothetical acid is possibly decomposed in digestion into free pepsin and free hydrochloric acid, which in *statu nascendi* dissolves proteids to a certain degree. The pepsin set free reunites with a new portion of acid, forming pepsin hydrochloric acid, and in contact with proteids is further decomposed as above described. It is hardly necessary to mention that this statement is only an unproved hypothesis.

Rennin or CHYMOSIN is the second enzyme of the gastric juice. It occurs in human gastric under physiological conditions, but may be absent under special pathological conditions, such as carcinoma, atrophy of the mucous membrane, and certain chronic catarrhs (BOAS, JOHNSON, KLEMPERER).[2] It is habitually found in the neutral, watery infusion of the fourth stomach of the calf and sheep, especially in an infusion of the fundus part. In other mammals and in birds it is seldom found, and in fishes hardly ever in the neutral infusion.

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[1] Zeitschr. f. Biologie, Bd. 28, also contains the literature.

[2] A good review of the literature may be found in Szydlowski, Beitrag zur Kenntniss des Labenzym nach Beobachtungen an Säuglingen, Jahrb. f. Kinderheilkunde, N. F., Bd. 34.

In these cases a rennin-forming substance, a *rennin zymogen*, occurs which is converted into rennin by the action of an acid.

Rennin is just as difficult to prepare in a pure state as the other enzymes. The purest rennin enzyme thus far obtained did not give the ordinary proteid reactions. On heating its solutions it is destroyed, and indeed more easily in acid than in neutral solutions. If an active and strong infusion of a mucous coat in water containing 3 p. m. HCl is heated to 37–40° C. for 48 hours, the rennin is destroyed, while the pepsin remains. A pepsin solution free from rennin can be obtained in this way. Rennin is characterized by its physiological action, which consists in coagulating milk or a casein solution containing lime, if neutral or very faintly alkaline.

Rennin may be carried down by other precipitates like other enzymes, and thus may be obtained relatively pure. It may also be obtained, contaminated with a great deal of proteids, by extracting the mucous coat of the stomach with glycerin.

A comparatively pure solution of rennin may be obtained in the following way. An infusion of the mucous coat of the stomach in hydrochloric acid is prepared and then neutralized, after which it is repeatedly shaken with new quantities of magnesium carbonate until the pepsin is precipitated. The filtrate, which should act strongly on milk, is precipitated by basic lead acetate, the precipitate decomposed with very dilute sulphuric acid, the acid liquid filtered and treated with a solution of stearin soap. The rennin is carried down by the fatty acids set free, and when these last are placed in water and removed by shaking with ether, the rennin remains in the watery solution.

A fasting animal may secrete a strongly-acid gastric juice. The acid of the gastric juice then cannot be derived from the foods, but must originate in the mucous coat. As the pyloric glands, which contain no parietal cells, secrete an alkaline secretion according to HEIDENHAIN[1] and KLEMENSIEWICZ,[2] while the fundus glands, which contain these cells, yield an acid secretion, it is generally assumed with HEIDENHAIN that the parietal cells are of special importance in the secretion of free hydrochloric acid —a statement which other observations tend to confirm. The later investigations of FRANKEL[3] and CONTEJEAN[4] seem to contradict this statement. They claim that the chief cells as well as the parietal cells take part in the formation of acid.

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[1] Pflüger's Arch., Bdd. 18 and 19. See also Hermann's Handbuch, Bd. 5, Th. 1, "Absonderungsvorgänge."

[2] Wien. Sitzungsber, Bd. 71.

[3] Pflüger's Arch., Bdd. 48 and 50.

[4] Contribution à l'étude de la physiol. de l'estomac, Thèse, Paris, 1892. Also Maly's Jahresber., Bd. 22, S. 293.

That the hydrochloric acid must originate from the chlorides of the blood is evident, and Kahn[1] has given a direct proof for this. He found in dogs that after a sufficiently long common-salt starvation that the stomach secreted a gastric juice containing pepsin but no free hydrochloric acid. On the administration of soluble chlorides a gastric juice containing hydrochloric acid was immediately secreted. We do not know how the secretion of free hydrochloric acid originates.

Whereas it used to be considered that the chlorides were decomposed by an electrolysis or by organic acids produced in the mucosa, we now rather generally accept the process as suggested by Maly.

Maly[2] has called attention to the fact that, on account of the presence of a large quantity of free carbon dioxide in the blood and the avidity of the same, there must be present among the numerous combinations of acids and bases which exist in the serum traces of free hydrochloric acid in addition to acid salts. As these traces of hydrochloric acid are removed from the blood by means of rapid diffusion by the glands, the mass-action of the carbon dioxide must set free new traces of hydrochloric acid in the blood. In this way may be explained the secretion in the blood of large quantities of hydrochloric acid from the chlorides, but the proof that the hydrochloric acid set free passes into the gastric juice simply by diffusion, is missing. Similar processes in other animal glands render it probable that here, as in other cases of secretion, we have to deal with a yet unexplained specific secretory action of the glandular cells.

L Liebermann[3] has lately proposed a new theory for the secretion of hydrochloric acid. According to him lecithalbumin occurs in the glandular cells, and this combines readily with alkalies. The more active metabolism in the glands during work leads to an abundant formation of carbon dioxide, and this carbon dioxide by its mass-action sets hydrochloric acid free from the chlorides. The hydrochloric acid passes into the secretion by diffusion, while the alkalies combine with the lecithalbumin. In regard to details of this theory we must refer the reader to the original article.

Nencki and Schoumow-Simanowsky[4] have confirmed, on dogs, theo bservations of Külz,[5] namely, that the introduction of alkali bromides or iodides causes a replacement of the hydrochoric acid of the gastric juice by HBr or to a smaller extent by HI. On the determination of the quantity of chlorine in the various tissues and fluids under normal conditions and after the administration of NaBr they have shown that bromine can also replace the chlorine in the organism.

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[1] Zeitschr. f. physiol. Chem., Bd. 10.

[2] *Ibid.*, 1.

[3] Pflüger's Arch., Bd. 50.

[4] Arch. des Sciences biol. de St. Petersboug, Tome 8.

[5] Zeitschr. f. Biologie, Bd. 23.

After an abundant meal, when the store of pepsin in the stomach is completely exhausted, SCHIFF claims that certain bodies, especially dextrin, have the property of causing a supply of pepsin in the mucous membrane. This "charge theory," though experimentally proved by several investigators, has nevertheless not yet been confirmed. On the contrary, the statement of SCHIFF[1] that a substance forming pepsin, a "*pepsinogen*" or "*propepsin*," occurs in the ventricle has been proved. LANGLEY[2] has shown positively the existence of such a substance in the mucous coat. This substance, propepsin, shows a comparatively strong resistance to dilute alkalies (a soda solution of 5 p. m.), which easily destroy pepsin (LANGLEY). Pepsin, on the other hand, withstands better than propepsin the action of carbon dioxide, which quickly destroys the latter. The occurrence of a rennin zymogen in the mucous coat has been mentioned above.

The question in which cells the two zymogens, especially the propepsin, are produced has been extensively discussed for several years. Formerly it was the general opinion that the parietal cells were pepsin cells, but since the investigations of HEIDENHAIN and his pupils, LANGLEY and others, the formation of pepsin has been shifted to the chief cells. FRÄNKEL and CONTEJEAN have lately presented objections to the views of HEIDENHAIN that certains cells produce the zymogens and others only the acid.

The Pyloric Secretion. That part of the pyloric end of the dog's stomach which contains no fundus glands was dissected by KLEMENSIEWICZ, one end being sewed together in the shape of a blind sack and the other sewed into the stomach. From the fistula thus created he was able to obtain the pyloric secretion of a living animal. This secretion is alkaline, viscous, jelly-like, rich in mucin, of a specific gravity of 1.009–1.010, and containing 16.5–20.5 p. m. solids. It has no effect on fat, but acts, though very slowly, on starch, converting it into sugar, and contains ordinarily pepsin, which sometimes occurs in considerable amounts. This has been observed by HEIDENHAIN in permanent pyloric fistula. CONTEJEAN[1] has investigated the pyloric secretion in other ways, and finds that it contains both acid and pepsin. The alkaline reaction of the secretions investigated by HEIDENHAIN and KLEMENSIEWICZ is due, according to CONTEJEAN, to an abnormal secretion caused by the operation, because the stomach readily yields an alkaline juice instead of an acid one under abnormal conditions. ÅKERMAN[2] has found, in accordance with HEIDENHAIN and KLEMENSIEWICZ, that the pyloric secretion of a dog was alkaline. He could never detect free acid, but always pepsin and rennin.

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[1] Leçons sur la physiol. de la digestion, 1867, Tome 2.

[2] Langley and Edkins, Journ. of Physiol., Vol. 7.

The secretion of the juice of the stomach is dependent to a great extent upon the excitement acting on the mucous coat of the stomach, and it follows from this that the quantity of secretion under different conditions must vary considerably. The statements of the quantity of gastric juice secreted in a certain time are therefore so unreliable that they need not be taken into account.

The Chyme and the Digestion in the Stomach. By means of the mechanical irritation of the mucous coat of the stomach, as well as by the chemical irritation caused by the food and saliva, an abundant secretion of gastric juice occurs. The food is thereby freely mixed with liquid and is gradually converted into a pulpy mass, called the chyme. This mass is acid in reaction, and, with the exception of the interior of large pieces of meat or other solid foods, the chyme is acid throughout. The transformation products of the digestion of proteids and carbohydrates can be detected in the chyme; likewise more or less changed undigested residues of swallowed food, which indeed form the chief mass of the chyme.

In the chyme morsels of MEAT more or less changed are found which, when unboiled meat is partaken of, may be much swollen and slippery. MUSCLE and CARTILAGE are also often swollen and slippery, while pieces of BONE sometimes show a rough and uneven surface after the digestion has continued for some time, which depends upon the fact that the gelatinous substances of the bone are attacked more quickly by the gastric juice than the earthy parts. MILK coagulates in the stomach by the combined action of the rennin and the acid, but in certain cases by the action of the acid alone. From the relative quantities of the swallowed milk to the other food either large and solid lumps of cheese are formed or smaller lumps or grains which are divided in the pulpy mass. Cow's milk regularly yields large, solid masses or lumps; human milk gives, on the contrary, a fine, loose coagulum or a fine precipitate which is immediately dissolved in part by the acid liquid. The milk-sugar may pass into lactic-acid fermentation, and this, according to RICHET, is the reason why the acid reaction of the contents of the stomach is greater at the end of the digestion of a meal consisting mainly of milk.

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[1] L. c.

[2] Skand. Arch. f. Physiol., Bd. 5.

BREAD, especially when not too fresh, is converted rather easily into a pulpy mass in the stomach. Other vegetable foods, such as POTATOES, may, if not sufficiently masticated, often be found in the contents of the stomach, very little changed, several hours after a meal.

STARCH is not converted into sugar by the gastric juice, but in the first phases of the digestion, before a large quantity of hydrochloric acid has accumulated, it seems that the action of the saliva continues, and therefore the presence of dextrin and sugar can be detected in the contents of the stomach. Besides this the carbohydrates in the stomach may in part undergo a lactic-acid fermentation, caused by the micro-organisms present.

According to the investigations of ELLENBERGER and HOFFMEISTER[1] on horses and pigs, after a meal rich in amylaceous bodies in the first phase of the digestion, an AMYLOLYSIS takes place with the formation of lactic acid; then gastric juice containing hydrochloric acid is secreted, when a second phase in which PROTEOLYSIS takes place. As a rule, the formation of lactic acid decreases as the secretion of hydrochloric acid increases. EWALD and BOAS[2] claim that a similar condition also exists in human beings. They claim that there is in the first stage of digestion a predominance of lactic acid in the stomach, in the second a simultaneous occurrence of lactic and hydrochloric acids, and in the third stage almost exclusively hydrochloric acids. KJAERGAARD[3] has lately formed the same conclusions from his investigations on children and robust persons. In persons with altered blood-vessels due to senility the contents of the stomach show chiefly the presence of lactic acid. Such persons digest large amounts of carbohydrates, while the digestion of albuminous bodies is decreased. From recent investigations, making use of his new method of estimating lactic acid, BOAS[1] considers that after partaking of carbohydrates lactic acid does not occur in the stomach under normal conditions nor during the continual lack of hydrochloric acid. Lactic acid, on the contrary, is regularly found in carcinoma.

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[1] Maly's Jahresber., Bdd. 15 and 16.

[2] Virchow's Arch., Bd. 101.

[3] Om. Ventrikelfordöjelsen hos sunde Mennesker, Kjöbenhavn, 1888. See Maly's Jahresber., Bd. 19.

The FATS which are not fluid at the ordinary temperature melt in the stomach at the temperature of the body and become fluid. In the same way the fat of the fatty tissues is set free in the stomach by the gastric juice which digests the cell-membrane. The gastric juice itself seems to have no action on fats.[2] The soluble salts of the food naturally are found dissolved in the liquids of the contents of the stomach; but the insoluble salts may also be dissolved by the acid of the gastric juice.

Since the hydrochloric acid of the gastric juice prevents the contents of the stomach from fermenting with the generation of gas, those *gases* which occur in the stomach probably depend, at least in great measure, upon the swallowed air and saliva, and upon those gases generated in the intestine and returned through the pyloric valve. PLANER[3] found in the stomach-gases of a dog 66–68% N, 25–33% CO_2, and only a small quantity, 0.8–6.1%, of oxygen. SCHIERBECK[4] has shown that a part of the carbon dioxide is formed by the mucous membrane of the stomach. The tension of the carbon dioxide in the stomach corresponds, according to him, to 30–40 mm. Hg in the fasting condition. It increases after partaking food, independently of the kind of food, and may rise to 130–140 mm. Hg during digestion. The curve of the carbon-dioxide tension in the stomach is the same as the curve of acidity in the different phases of digestion, and SCHIERBECK has also found that the carbon-dioxide tension is considerably increased by pilocarpin, but diminished by nicotin. According to him, the carbon dioxide of the stomach is a product of the activity of the secretory cells.

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[1] Berlin. klin. Wochenschr., 1895.

[2] See Contejean, "Sur la digestion gastrique de la graisse," Arch. de Physiologie, (5) Bd. 6.

[3] Wien. Sitzungsber., Bd. 42, 1860.

[4] Skan. Arch. f. Physiol., Bdd. 3 and 5.

According as the food is finely or coarsely divided it passes sooner or later through the pylorus into the intestine. From BUSCH'S[1] observations on a human intestinal fistula, it required generally 15–30 minutes after eating for undigested food, such as pieces of meat, to pass into the upper part of the small intestine. In a case of duodenal fistula in a human being observed by KÜHNE,[2] he saw, ten minutes after eating, uncurdled but still coagulable milk and small pieces of meat pass out of the fistula. The time in which the stomach unburdens itself of its contents depends, however, upon the rapidity with which the quantity of hydrochloric acid increases, for it seems to act as a sort of irritant and causes the opening of the pylorus. Many other conditions also come into play, namely, the activity of the gastric juice, the quantity and character of the food, etc., etc., and therefore the time required to empty the stomach must be variable. RICHET[3] observed in a case of stomachic fistula that in man the quantity of food which is in the stomach the first three hours is not essentially changed, but that in the course of a quarter of an hour nearly all is driven out, so that only a small residue remains. KÜHNE[4] has made about the same observations on dogs and human beings. He found, indeed, in dogs that in the first hour small quantities of meat passed into the intestine every ten minutes; but he also observed that in dogs, on an average, about five hours after eating, in man somewhat earlier, a free emptying into the intestine takes place. According to other investigators, the emptying of the human stomach does not take place suddenly, but gradually. BEAUMONT[5] found in his extensive observations on the Canadian hunter, ST. MARTIN, that the stomach, as a rule, is emptied 1½–5½ hours after a meal, depending upon the character of the food.

The time in which different foods leave the stomach depends also upon their digestibility. In regard to the unequal digestibility in the stomach of foods rich in proteids, which really form the object of the action of the gastric juice, a distinction must be made between the rapidity with which the proteids are converted into albumoses and peptones and the rapidity with which the food is converted into chyme, or at least so prepared that it may easily pass into the intestine. This distinction is especially important from a practical standpoint. When a proper food is to be decided upon in cases of diminished stomachic digestion, it is important to select such foods as, independent of the difficulty or ease with which their proteid is peptonized, leave the stomach easily and quickly, and which require as little action as possible on the part of this organ. From this point of view those foods, as a rule, are most digestible which are fluid from the start or may be easily liquefied in the stomach; but these foods are not always the most digestible in the sense that their proteid is most easily peptonized. As an example, hard-boiled white of egg is more easily peptonized than fluid white of egg at a degree of acidity of 1–2 p. m. HCl;[1] nevertheless we consider, and justly, that an unboiled or soft-boiled egg is easier to digest than a hard-boiled one. Likewise uncooked meat, when it is not chopped very fine, is not more quickly but more slowly peptonized by the gastric juice than the cooked, but if it is divided sufficiently fine it is often more quickly peptonized than the cooked.

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[1] Virchow's Arch., Bd. 14.

[2] Lehrbuch d. physiol. Chem., S. 53.

[3] L. c.

[4] *Ibid.*

[5] *Ibid.*

The greater or less facility with which the different albuminous foods are peptonized by the gastric juice has been comparatively little studied, and as the conditions in the stomach are more complicated, results obtained with artificial gastric juice are often of no value for the practising physician and should in any case be used only with the greatest caution. Under these circumstances we cannot enter more deeply into this subject, but the reader is referred to text-books on dietetics and the study of foods.

As our knowledge of the digestibility of the different foods in the stomach is slight and dubious, so also our knowledge of the action of other bodies, such as alcoholic drinks, bitter principles, spices, etc., on the natural digestion is very uncertain and imperfect. The difficulties which stand in the way of this kind of investigation are very great, and therefore the results obtained thus far are often ambiguous or conflict with each other. For example, certain investigators have observed that small quantities of alcohol or alcoholic drinks do not prevent but rather facilitate digestion; others observe only a disturbing action; while other investigators believe to have found that the alcohol first acts somewhat as a disturbing agent, but afterwards, when it is absorbed, it produces an abundant secretion of gastric juice, and thereby facilitates digestion (GLUZINSKI,[1] CHITTENDEN [2]).

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[1] Wawrinsky, Upsala Läkarefs. Förh., Bd. 8; see also Maly's Jahresber., Bd. 8.

The digestion of sundry foods is not dependent on one organ alone, but divided among several. For this reason it is to be expected that the various digestive organs can act for one another to a certain point, and that therefore the work of the stomach could be taken up more or less by the intestine. This in fact is the case. Thus the stomach of a dog has been almost completely extirpated (CZERNY,[3] CARVALLO, and PANCHON [4]), and also that part necessary in the digestive process has been eliminated by plugging the pyloric opening (LUDWIG and OGATA [5]), and in both cases it was possible to keep the animal alive, well fed, and strong. In these cases it is evident that the digestive work of the stomach was taken up by the intestine. That the stomach nevertheless, during normal conditions, bears an essential part of the process of digestion may be inferred from the fact that the products of proteolysis can generally be detected in the contents of the human stomach even shortly after a meal. By tests on dogs that had been given meat-powder, CAHN [6] found large quantities of peptone in the stomach, and this progressed to the same extent as the digestion, although absorption took place, as shown by SCHMIDT-MÜLHEIM.[7]

It is, however, quite generally assumed that no peptonization of the proteids worth mentioning occurs in the stomach, and that the albuminous foods are only prepared in the stomach for the real digestive processes in the intestine. That the stomach serves in the first place as a storeroom follows from its shape, and this function is of special value in certain new-born animals, for instance in dogs and cats. In these animals the secretion of the stomach contains only hydrochloric acid but no pepsin, and the casein of the milk is converted by the acid alone into solid lumps or a solid coagulum which fills the stomach. Small portions of this coagulum pass in to the intestine only little by little, and an overburdening of the intestine is thus prevented. In other animals, such as the snake and certain fishes, which swallow their food entire, it is certain that the major part of the process of digestion takes place in the stomach. The importance of the stomach in digestion cannot at once be decided. It varies for different animals, and it may vary in the same animal, depending upon the division of the food, the rapidity with which the peptonization takes place, the more or less rapid increase in the amount of hydrochloric acid, and so on.

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[1] Deutsch. Arch. f. klin. Med., Bd. 39.

[2] Centralbl. f. d. med. Miss., 1889, S. 435.

[3] Czerny, Beiträge zur operativen Chirurgie. Stuttgart, 1878. Cited from Bunge, Lehrbuch der physiol u. path. Chem., p. 150.

[4] Arch. de physiol., (5) Tome 7, p. 106.

[5] Du Bois-Reymond's Arch, 1883.

[6] Zeitschr. f. klin. Med., Bd. 12.

[7] Du Bois-Reymond's Arch., 1879, S. 39.

It is a well-known fact that the contents of the stomach may be kept without decomposing for some time by means of hydrochloric acid, while, on the contrary, when the acid is neutralized a fermentation commences by which lactic acid and other organic acids are formed. The hydrochloric acid of the gastric juice has unquestionably an anti-fermentive[1] action, and also, like dilute mineral acids, an antiseptic action. This action is of importance, as many disease micro-organisms may be destroyed by the gastric juice. The comma bacillus of cholera is killed by the normal acid gastric juice, while if it is introduced into the stomach after an injection of a soda solution it may remain active. Also varieties of pyogenic streptococcus and the staphylococcus pyog. aureus are killed by the acid gastric juice. Still the gastric juice does not act on all micro-organisms, and especially in the state of spores they can withstand its action. As an example, the tubercle-virus is not destroyed by the gastric juice, and the spores of the anthrax bacteria are not always destroyed by the hydrochloric acid of the gastric juice.[2]

Because of this action[3] the chief importance of the gastric juice is now considered to be its antiseptic action. In opposition to this view CARVALLO and PACHON have shown in a dog with extirpated stomach that putrefying meat could be partaken of without disturbing the digestion.

After death, if the stomach still contains food, digestion goes on of itself not only in the stomach, but also in the neighboring organs, during the slow cooling of the body. This leads to the question, why does the stomach not digest itself during life? Ever since

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[1] See Kühne's Lehrbuch, p. 57; Bunge, Lehrbuch, pp. 142 and 152; F. Cohn, Zeitschr. f. physiol. Chem., Bd. 14; Hirschfeld, Pflüger's Arch., Bd. 47.

[2] See Falk, Virchow's Arch., Bd. 93; E. Frank, Deutsch. med. Wochenschr., 1884, No. 24; R. Koch, *ibid.*, 1884, No. 45.

[3] Bunge, l. c.

PAVY[1] has shown that after tying the smaller blood-vessels of the stomach of dogs the corresponding part of the mucous membrance was digested, efforts have been made to find the cause in the neutralization of the acid of the gastric juice by the alkali of the blood. That the reason for the non-digestion during life is to be sought for in the normal circulation of the blood cannot be contradicted; but it is more probably found in the fact that the living mucous coat nourished by the alkaline blood shows quite different absorption, diffusion, and filtration properties than the dead mucous coat. This last was shown long ago by RANKE.[2]

Under pathological conditions irregularities in the secretion as well as in the absorption and in the mechanical work of the stomach may occur. Pepsin is almost always present, but the absence of the rennin, as above stated, may occur in many cases (BOAS, JOHNSON, KLEMPERER[3]). In regard to the acid, it should be mentioned that sometimes this secretion may be increased so that an abnormally acid gastric juice is secreted, and sometimes may be decreased so that little or hardly any hydrochloric acid is secreted. A hypersecretion of acid gastric juice sometimes occurs. In the secretion of too little hydrochloric acid the same conditions appear as after the neutralization of the acid contents of the stomach outside of the organism. Fermentation processes now appear in which, besides lactic acid, there appear also volatile fatty acids, such as butyric and acetic acids, etc., and gases like hydrogen. These fermentation products are therefore often found in the stomach in cases of chronic catarrh of the stomach, which may give rise to belching, pyrosis, and other symptoms.

Among the foreign substances found in the contents of the stomach we have UREA, or ammonium carbonate derived therefrom in uræmia; BLOOD, which generally forms a dark-brown mass through the presence of hæmatin, due to the action of the gastric juice; BILE, which, especially during vomiting, easily finds its way through the pylorus into the stomach, but whose presence seems to be without importance.

If it is desired to test the gastric juice or the contents of the stomach for *pepsin*, fibrin may be employed. If this is thoroughly washed immediately after beating the blood, well pressed and placed in glycerin, it may be kept in serviceable condition an indefinitely long time. The gastric juice or the matter contained in the stomach—the latter, if necessary, having been previously diluted with 1 p. m. hydrochloric acid—is filtered and tested with fibrin at ordinary temperature. (It must not be forgotten that a control test must be made with acid alone and another portion of the same fibrin.) If the fibrin is not noticeably digested within one or two hours, no pepsin is present, or at most there are only slight traces.

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[1] Philos. Transactions, Vol. 153, Part 1, and Guy's Hospital Reports, Vol. 13.

[2] See Ranke, Grundzüge der Physiol., 3. Aufl., 1875, S. 111-120.

[3] See foot-note, p. 272.

In testing for *rennin* the liquid must be first carefully neutralized. To 10 c. c. unboiled amphoteric (not acid) reacting cow's milk add 1–2 c. c. of the filtered neutralized liquid; but care must be taken not to add too much of the liquid from the stomach, for the coagulation may be retarded or prevented by diluting the milk. In the presence of rennin the milk should coagulate to a solid mass at the temperature of the body in the course of 10–20 minutes without changing its reaction. If the milk is diluted too much by the addition of the liquid of the stomach, only coarse flakes are obtained and no solid coagulum. Addition of lime-salts is to be avoided, as they in great excess may produce a partial coagulation even in the absence of rennin.

In many cases it is especially important to determine the *degree of acidity of the gastric juice*. This may be done by the ordinary titration methods. Phenol phthalein must not be used as an indicator, for we get too high results in the presence of large quantities of proteids. Good results may be obtained, on the contrary, by using very delicate litmus-paper. As the acid reaction of the contents of the stomach may be caused simultaneously by several acids, still the degree of acidity is here, as in other cases, expressed in only one acid, e.g., HCl. Generally the acidity is expressed by the number of c. c. of $\frac{N}{10}$ caustic soda which is required to neutralize the several acids in 100 c. c. of the liquid of the stomach. An acidity of 43% means that 100 c. c. of the liquid of the stomach required 43 c. c. of $\frac{N}{10}$ caustic soda to neutralize it.

The acid reaction may be partly due to free acid, partly to acid salts (monophosphates), and partly to both. According to LEO[1] we can test for acid phosphates by calcium carbonate, which is not neutralized therewith, while the free acids are. If the gastric content has a neutral reaction after shaking with calcium carbonate and the carbon dioxide is driven out by a current of air, then it contains only free acid; if it has an acid reaction, then acid phosphates are present; and if it is less acid than before, it contains both free acid and acid phosphate. This method can also be applied in the estimation of free acid (see below).

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[1] Centralbl. f. d. med. Wissensch., 1889, S. 481, and Diagnostik der Krankheiten der Verdauungsorgane (Berlin, 1890), also Pflüger's Arch., Bd. 48, S. 614.

It is also important to be able to ascertain the nature of the acid or acids occurring in the contents of the stomach. For this purpose, and especially for the *detection of free hydrochloric acid*, a great number of color reactions have been proposed, which are all based upon the fact that the coloring substance gives a characteristic color with very small quantities of hydrochloric acid, while lactic acid and the other organic acids do not give these colorations, or only in a certain concentration, which can hardly exist in the contents of the stomach. These reagents are a mixture of FERRIC ACETATE and POTASSIUM SULPHOCYANIDE solution (MOHR's reagent has been modified by several investigators), METHYLANILIN-VIOLET, TROPÆOLIN 00, CONGO RED, MALACHITE-GREEN, PHLOROGLUCIN-VANILLIN, BENZOPURPURIN 6 B, and others. As reagents for *free lactic acid* UFFELMANN suggests a strongly diluted, amethyst-blue solution of FERRIC CHLORIDE and CARBOLIC ACID or a strongly diluted, nearly colorless solution of FERRIC CHLORIDE. These give a yellow with lactic acid, but not with hydrochloric acid or with volatile fatty acids. Instead of the untrustworthy lactic-acid reaction of UFFELMANN, BOAS[1] makes use, in the detection and estimation of lactic acid, of the property of lactic acid of being oxidized into aldehyde and formic acid on careful oxidation with sulphuric acid and manganese dioxide. The aldehyde is detected by its forming iodoform with an alkaline iodine solution or by its forming aldehyde mercury with NESSLER's reagent. The quantitative estimation consists in the formation of iodoform with $\frac{N}{10}$ iodine solution and caustic potash, adding an excess of hydrochloric acid and titrating with a $\frac{N}{10}$ sodium arsenite solution and retitrating with iodine solution, after the addition of starch-paste, until a blue coloration is obtained.

The value of these reagents in testing for free hydrochloric acid or lactic acid is still disputed.[2] Among the reagents for free hydrochloric acid, MOHR's test (even though not very delicate), GUNZBURG's test with phloroglucin-vanillin, and the test with tropæolin 00, performed in moderate heat as suggested by BOAS, seem to be the most valuable. If these tests give positive results, then the presence of hydrochloric acid may be considered as proved. A negative result does not eliminate the presence of hydrochloric acid, as the delicacy of these reactions has a limit, and also the simultaneous presence of proteid, peptones, and other bodies influences the reactions more or less. The reactions for lactic acid may also give negative results in the presence of comparatively large quantities of hydrochloric acid in the liquid to be tested. Sugar, sulphocyanides, and other bodies may act with these reagents similarly to lactic acid.

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[1] Deutsch. med. Wochenschr., 1893, and Münchener med. Wochenschr., 1893.

[2] In regard to the extensive literature on this question we refer to v. Jaksch, Klinische Diagnostik innerer Krankheiten, 4. Aufl., 1896, Section 5.

In order to be able to correctly judge of the value of the different reagents for free hydrochloric acid, it is naturally of greatest importance to be clear in regard to what we mean by free hydrochloric acid. It is a well-known fact that hydrochloric acid combines with proteids, and a considerable part of the hydrochloric acid may therefore exist in the contents of the stomach, after a meal rich in proteids, in combination with proteids. This hydrochloric acid combined with proteids, as well as that which is combined with amido-acids, cannot be considered as free, and it is for this reason that certain investigators consider such methods as those of LEO and SJÖQVIST, which will be described below, as of little value. However, it must be remarked that, according to the unanimous experience of many investigators, the hydrochloric acid combined with proteids and also that combined with amido-acids (SALKOWSKI and KUMAGAWA[1]) is physiologically active. Those reactions (color reactions) which only respond to actually free hydrochloric acid do not show the physiologically active hydrochloric acid. The suggestion of determining the "physiologically active" hydrochloric acid instead of the "free" seems to be correct in principle; and as the conceptions of free and physiologically active hydrochloric acid do not cover one another we must always be clear, if we want to determine the actually free or the physiologically active hydrochloric acid, before we judge of the value of a certain reaction.

As the above-mentioned reactions for hydrochloric acid and organic acids are not sufficient in exact investigations, still they may serve in many cases for clinical purposes, and it will suffice to refer the reader to other text-books, and especially to "*Klinische Diagnostik innerer Krankheiten*," by R. v. JAKSCH, 4th edition, 1896, for the performance and the relative value of these tests.

Among the many methods suggested for the quantitative estimation of hydrochloric acid not combined with inorganic bases, the two following are the most trustworthy.

The *method of K. MORNER and SJÖQVIST* depends on the following principle: When the gastric juice is evaporated to dryness with barium carbonate and then calcined the organic acids burn up and give insoluble barium carbonate, while the hydrochloric acid forms soluble barium chloride. From the quantity of this the original amount of hydrochloric acid can be calculated. 10 c. c. of the filtered contents of the stomach is mixed in a small platinum or silver dish with a knife-point of barium carbonate free from chlorides, and evaporated to dryness. The residue is burnt and allowed to glow for a few minutes. The cooled carbon is gently rubbed with water and completely extracted with boiling water, and the filtrate (about 50 c. c.) treated with an equal volume of alcohol and 3–4 c. c. sodium acetate solution (10% acetic acid and 10% acetate). The amount of barium in the filtrate is determined by titration with a solution of potassium bichromate, in which the alcohol facilitates the precipitation of the barium chromate, while the acetate prevents in part the precipitation of the calciuum carbonate and in part the setting free of hydrochloric acid. The potassium-bichromate solution should contain about 8.5 grms. potassium bichromate to the litre. Its titre must exactly correspond with an $\frac{N}{10}$ barium-chloride solution, and the procedure is the same as in the titration of the $BaCl_2$ solution obtained from the contents of the stomach. A paper moistened with tetramethylparaphenylendiamin is used as indicator; this is colored blue by a bichromate in acetic-acid solution. In titrating we add chromate solution as long as the barium chromate precipitated does not apparently increase, then test with the indicator-paper after each addition until it gives a decided blue coloration within one minute, and stop adding chromate solution. As the titre of the chromate solution has been determined by an $\frac{N}{10}$ $BaCl_2$ solution, it is easy to calculate the quantity of HCl in 10 c. c. of the gastric juice corresponding to the number of c. c. of the chromate solution used. If the total acidity is determined in a second portion of the gastric juice, then the quantity of lactic acid or other organic acids represented as HCl may be calculated. v. Jaksch suggests precipitating the barium with sulphuric acid and weighing the sulphate instead of titrating. Sjöqvist[1] has modified his method of determining hydrochloric acid by precipitating the solution of $BaCl_2$ by ammonium chromate in the presence of acetic acid. This precipitate is dissolved in water by the aid of a little HCl, then titrated with a potassium-iodide solution and hydrochloric acid, and now titrated with sodium hyposulphite. The reactions take place as follows: $4HCl + 2BaCO_3 = 2BaCl_2 + 2H_2O + 2CO_2$; $2BaCl_2 + 2(NH_4)_2CrO_4 = 2BaCrO_4 + 4NH_4Cl$; $2BaCrO_4 + 16HCl + 6KI = 2BaCl_2 + Cr_2Cl_6 + 8H_2O + 6KCl + 3I_2$; and $3I_2 + 6Na_2S_2O_3 = 6NaI + 3Na_2S_4O_6$. Each c.c. of the hyposulphite corresponds to 3 mgm. HCl. Other modifications of this method have been proposed by Salkowski and Fawitzki,[2] Boas,[3] and Bourget.[4] This method of Mörner-Sjöqvist gives, according to Leo[5] and Koss-

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[1] Virchow's Arch., Bd. 122.

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[1] Skan. Arch. f. Physiol., Bd. 5.

[2] Virchow's Arch., Bd. 123.

[3] Centralbl. f. klin. Med., Bd. 12.

[4] Schmidt's Jahrbücher, 1891, Bd. 229 (Reference).

[5] L. c.

LER,[1] in the presence of phosphates, too low values, but it is otherwise very good.

LEO'S Method.[2] 10 c. c. of the filtered gastric juice is treated with about 5 c. c. calcium-chloride solution, and the total acidity determined by $\frac{N}{10}$ caustic-soda solution, using litmus as the indicator. Then shake 15 c. c. of the same gastric juice with pure, finely powdered calcium carbonate, filter through a dry filter, remove the carbon dioxide from the filtrate by means of a current of air, measure off exactly 10 c. c. of the liquid and treat with 5 c. c. of the calcium-chloride solution, and add litmus and titrate again. The difference between the two titrations shows the acidity due to free acid. Any fatty acids present may be shaken out from another portion by ether and the acidity determined on the spontaneous evaporation of the ether.

By determining the electrical resistance SJÖQVIST has been able to determine the amount of actually free acid and that combined with alkali in a mixture of hydrochloric acid and alkali monophosphate. He finds that the quantity of hydrochloric acid found by MÖRNER-SJÖQVIST'S method in such mixtures corresponds very closely to the quantity actually present. He upholds his method in opposition to LEO'S method, which, according to him, does not give accurate results for free acid.

Other methods have been proposed by CAHN and V. MERING, HOFFMANN, WINTER and HAYEM, and BRAUN. According to KOSSLER[3] the three last-mentioned methods are not quite serviceable.

In testing for *volatile fatty acids* the contents of the stomach should not be directly distilled, as volatile fatty acids may be formed by the decomposition of other bodies, such as proteid and hæmoglobin. The neutralized contents of the stomach are therefore precipitated with alcohol at ordinary temperature, filtered quickly, pressed, and repeatedly extracted with alcohol. The alcoholic extracts are made faintly alkaline by soda, and the alcohol distilled. The residue is now acidified by sulphuric or phosphoric acid and distilled. The distillate is neutralized by soda and evaporated to dryness on the water-bath. The residue is extracted with absolute alcohol, filtered, the alcohol distilled off, and this residue dissolved in a little water. This solution may be directly tested for acetic acid with sulphuric acid and alcohol or with ferric chloride. Formic acid may be tested for by silver nitrate, which quickly gives a black precipitate; and butyric acid is detected by the odor after the addition of an acid. In regard to the methods for more fully investigating the different volatile fatty acids, the reader is referred to other text-books.

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[1] Zeitschr. f. physiol. Chem., Bd. 17.

[2] L. c.

[3] L. c.; see also Mizerski and L. Nencki, Arch. des sciences biologiques, St. Pétersbourg, Tome 1.

III. The Glands of the Mucous Membrane of the Intestine and their Secretions.

The Secretion of Brunner's Glands. These glands are partly considered as small pancreas glands and partly as mucous or salivary glands. Their importance in various animals is different. According to GRÜTZNER,[1] in dogs they are closely related to the pyloric glands and contain pepsin. The statements in regard to the occurrence of a diastatic enzyme are contradictory, and the difficulty of collecting the secretion from these glands free from contamination makes these assumptions somewhat unreliable.

The Secretion of Lieberkuhn's Glands. The secretion of these glands has been studied by the aid of a fistula in the intestine according to the method of THIRY[2] and VELLA.[3] Very little if any secretion takes place in fasting animals (dog) when the mucous membrane is not irritated. The secretion begins in the first hour after partaking of food, but the maximum varies with the quantity and character of the food.[4] Mechanical, chemical, or electrical irritation excites a secretion or increases that already begun (THIRY). Laxatives do not increase the secretion, while pilocarpin produces a very abundant one (MASLOFF[5] and VELLA). The quantity of this secretion in the course of 24 hours has not been exactly determined.

In the upper part of the small intestine of the dog this secretion is scanty, slimy, and gelatinous; in the lower part it is more fluid, with gelatinous lumps or flakes (RÖHMANN[6]). Intestinal juice has a strong alkaline reaction, generates carbon dioxide on the addition of an acid, and contains (in dogs) nearly a constant quantity of $NaCl$ and Na_2CO_3, 4.8–5 and 4–5 p. m. respectively (GUMILEWSKI,[7] RÖHMANN). It contains proteid (THIRY found 8.01 p. m.), the quantity decreasing with the duration of the elimination. The

[1] Pflüger's Arch., Bd. 12.

[2] Wien. Sitzungsber., Bd. 50.

[3] Moleschott's Untersuch., Bd. 13.

[4] See Heidenhain in Hermann's Handbuch, Bd. 5, Th. 1, S. 170.

[5] Cited from Heidenhain, *ibid.*, S. 171.

[6] Pflüger's Arch., Bd. 41.

[7] *Ibid.*, Bd. 39.

quantity of solids varies. In dogs the quantity of solids is 12.2–24.1 p. m., and in sheep 46–47 p. m. The specific gravity of the intestinal juice of the dog, according to the observations of THIRY, is 1.010–1.0107.

The action of the intestinal juice has been studied by many investigators, but the statements concerning it are at variance. According to certain experimenters it has the power of converting starch into sugar, but others claim that it has not the property. However, it seems generally accepted, as shown by PASCHUTIN,[1] BROWN and HERON,[2] BASTIANELLI,[3] and others, that the intestinal juice or an infusion of the mucous membrane has an inverting action on cane-sugar or maltose. This has been further confirmed by MIURA, PAWTZ and VOGEL.[4] Lactose does not seem to be inverted by the intestinal juice in the absence of micro-organisms.[5] The action on carbohydrates takes place more quickly and to a greater extent in the upper part of the intestine, and correspondingly the absorption of starch and sugar occurs more quickly in the upper part than in the lower section of the intestine (LANNOIS and LÉPINE,[6] RÖHMANN).

Intestinal juice does not split neutral fats, but it has the property, like other alkaline fluids, of *emulsifying the fats.* In regard to its action on albuminous bodies most investigators agree that the intestinal juice has no action on boiled proteid or meat, while it *dissolves fibrin* according to THIRY. *Albumoses* are not converted into peptones (WENZ,[7] BASTIANELLI). Contrary to other investigators, SCHIFF[8] claims that the juice from a successful fistula operation digests not only coagulated proteid and lumps of casein, but also unboiled and boiled meat. The lack of action on proteids which was observed by other investigators SCHIFF attributes to the abnormal juice with which they experimented. SCHIFF also obtained from an operation not entirely successful a juice whose action on proteid and meat was no greater than that studied by THIRY and other investigators.

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[1] Centralbl. f. d. med. Wissensch., 1870, S. 561.

[2] Annal. d. Chem. u. Pharm., Bd. 204.

[3] Moleschott's Untersuch. zur Naturlehre, Bd. 14. This contains all the older literature.

[4] Zeitschr. f. Biologie, Bd. 32.

[5] Voit and Lusk, Zeitschr. f. Biologie, Bd. 28.

[6] Arch. de Physiol. (3) Tome 1.

[7] Zeitschr. f. Biologie, Bd. 22.

[8] Centralbl. f. d. med. Wissensch., 1868, S. 357.

Human intestinal juice in a case of *anus præternaturalis* has been investigated by DEMANT.[1] This juice showed itself entirely inactive on albuminous bodies, even on fibrin and on fats. It only had a very faint action on boiled starch. TUBBY and MANNING[2] have investigated human intestinal juice. The specific gravity was on an average 1.0069. The reaction was alkaline, and an abundant development of carbon dioxide took place on adding acid. Proteids were not digested; starch was first saccharified very slowly, while cane-sugar and maltose were inverted by the juice. Fats were both emulsified and saponified. These experiments on the action of the intestinal juice on food introduced into the intestine in cases of isolated loop of the intestine in animals, and in human intestine in cases of *anus præternaturalis*, have not given any positive results, because of the putrefaction processes going on in the intestine.

The secretion of the **glands in the large intestine** seems to consist chiefly of mucus. Fistulas have also been introduced into these parts of the intestine, which are chiefly if not entirely to be considered as absorption organs. The investigations on the action of this secretion on nutritive bodies have not as yet yielded any positive results.

IV. Pancreas and Pancreatic Juice.

In invertebrates, which have no pepsin digestion and which also have no formation of bile, the pancreas, or at least an analogous organ, seems to be the essential digestion gland. On the contrary, an anatomically characteristic pancreas is absent in certain vertebrates and in certain fishes. Those functions which should be performed by this organ seem to be performed in these animals by the liver, which may be rightly called HEPATOPANCREAS. In man and in most vertebrates the formation of bile and of certain secretions containing enzymes important for digestion is divided between the two organs, the liver and the pancreas.

The **pancreas gland** is similar in certain respects to the parotid gland. The secreting elements of the former consist of nucleated cells whose basis forms a mass rich in proteids, which expand in water and in which two distinct zones exist. The outer zone is more homogeneous, the inner cloudy due to a quantity of granules. The nucleus lies about midway between the two zones, but this position may change with the varying relative size of the two zones. According to HEIDENHAIN,[1] the inner part of the cells diminishes in size during the first stages of digestion, in which the secretion is active, while at the same time the outer zone enlarges owing to the absorption of new material. In a later stage, when the secretion has decreased and the absorption of the nutritive bodies has taken place, the inner zone enlarges at the expense of the outer, the substance of the latter having been converted into that of the former. Under physiological conditions the glandular cells are undergoing a constant change, at one time consuming from the inner part and at another time growing from the outer part. The inner granular zone is converted into the secretion, and the outer, more homogeneous zone, which contains the repairing material, is then converted into the granular substance.

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[1] Virchow's Arch., Bd. 75.

[2] Guy's Hosp. Report, Vol. 48, p. 277; also Centralbl. f. d. med. Wissensch., 1892, S. 945.

Besides considerable quantities of proteids, *globulin*, *nucleoproteid* (see Chapter II), and *albumin*, we find in this gland several enzymes, or, more correctly, *zymogens*, which will be described later. We also find in this gland *nuclein*, *leucin* (butalanin), *tyrosin* (not in the perfectly fresh gland), *xanthin*, 1–8 p. m., *hypoxanthin*, 3–4 p. m., *guanin*, 2–7.5 p. m. (all figures are calculated for the dried substance, KOSSEL[2]), *adenin*, *inosit*, *lactic acid*, *volatile fatty acids*, *fats*, and *mineral substances*. According to the investigations of OIDTMANN,[3] the human pancreas contains 745.3 p. m. water, 245.7 p. m. organic and 9.5 p. m. inorganic substances.

The purpose of the pancreas is to produce very important enzymes for digestion; but besides this it also has another very important function. As already stated in a preceding chapter, it is of the greatest importance in metabolism, namely, for the transformation of dextrose in the animal body. In this regard it is well known that in dogs and certain other animals (but not in pigeons and geese), the extirpation of the gland causes a marked diabetes, at least in most cases. We do not know how this diabetes is brought about.

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[1] Pflüger's Arch., Bd. 10.

[2] Zeitschr f. physiol. Chem., Bd. 8.

[3] v Gorup-Besanez, Lehrbuch, 4. Aufl., S. 732.

According to the brothers CAVAZZANI,[1] the pancreas diabetes is not caused by a decreased combustion of the normal quantity of sugar formed, but by an abnormal increase in the formation of sugar in the liver, and the extirpation of the pancreas acts, according to them, by causing a lesion of the plexus cœliacus. They have found that irritation of this plexus produced an increased production of sugar in the liver, and they claim that the extirpation of the pancreas induces a degenerative irritation of the plexus, which is similar to the paralytic secretion in the salivary glands. In opposition to this view the investigations of MINKOWSKI, HÉDON, LANCEREAUX, THIROLOIX,[2] and others have been presented, namely, that on subcutaneously transplanting a portion of the pancreas the function of the pancreas in transforming or producing sugar is not disturbed. After the removal of the intra-abdominal portion of the gland the animal in this case did not acquire diabetes. If the subcutaneously enveloped portion of pancreas is further removed, then an elimination of sugar of great intensity takes place.

CHAUVEAU and KAUFMANN[3] are of the opinion that after the extirpation of the pancreas an abnormal increase in the formation of sugar in the liver takes place. The pancreas, according to them, regulates the formation of sugar in the liver by means of two nerve-centres, a retarding and an irritating centre. The pancreas irritates the retarding centre and retards the irritating centre of the liver, and it has a double action on retarding the sugar production. The extirpation of the pancreas removes the irritation of the retarding centres, the activity of the irritating centres is thereby raised, and in consequence a strong hyperglycæmia takes place. In consideration of the above-mentioned action of transplanted pieces of pancreas, we must accept in these cases that the irritating action on the questionable centres under normal conditions is exercised by some other unknown internal secretory products of the gland.

The ordinary view in regard to the origin of diabetes is, however, as above (Chapter VIII) stated, that we have not to do with an increased production of sugar, but more likely a decreased transformation of the sugar in the animal body. We must also admit that the pancreas has the ability, in some way or other, of regulating the consumption of sugar; but we do not know how it acts. LÉPINE[1] has made an experiment to explain this action. According to him a glycolysis regularly takes place in the blood (see Chapter VI), and the enzyme active in this change is secreted from the pancreas to the blood. On the extirpation of the pancreas naturally this function of the gland is removed, hence a hyperglycæmia is produced. Important exceptions have been made against this hypothesis by several investigators,[2] and the action of the pancreas in the elimination of sugar still stands unexplained.

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[1] See Centralbl. f. Physiol., Bd. 7, S. 217.

[2] See Minkowski, Arch. f. exp. Path. u. Pharm., Bd. 31.

[3] Mem. Soc. Biol., 1893, p. 29. Cited from Centralbl. f. Physiol., Bd. 7, S. 317.

According to CHAUVEAU and KAUFMANN[3] a formation of sugar takes place in the liver, partly from the glycogen and partly from other bodies—carbohydrates, proteids, and fats—which on the destruction of tissues, the histolysis, are taken up by the blood and carried to the liver, where they are transformed into sugar. The pancreas has a preventive action on the sugar production of the liver, as also on the histolysis. This is caused by means of an unknown product of the inner secretion, which product passes into the blood. All three factors, the sugar production in the liver as well as the inner secretion of the pancreas and the histolysis, are, according to KAUFMANN, influenced in a double way by the nervous system, namely, partly exciting and partly retarding. The exciting action on the liver and on histolysis has simultaneously a preventive action on the internal secretion of the pancreas, and this therefore causes an increased formation of sugar in a threefold way. The preventive action on the liver and histolysis causes a simultaneous excitation of the internal secretion of the pancreas, and the formation of sugar under these conditions is reduced for three reasons. MARCUSE[4] has found that in frogs, in which ALDEHOFF has shown that diabetes may be produced on the extirpation of the pancreas, no diabetes appears on as perfect extirpation of the liver as possible.

Pancreatic Juice. This secretion may be obtained by adjusting a fistula in the excretory duct, according to the methods suggested by BERNARD,[5] LUDWIG,[6] and HEIDENHAIN.[7] If the operation is performed with sufficient rapidity and dexterity on an animal which has been well fed a few hours before, there is obtained from the fistula, as a rule, immediately after the operation (*temporary fistula*) a secretion rich in solids, viscid, very active, and which may be considered as normal pancreatic juice. Ordinarily, however, the gland becomes diseased in a few hours or days after the operation, and the secretion which then flows out of the fistula (*permanent fistula*) is more liquid, deficient in solids, and in certain other respects different from the secretion obtained immediately after the operation. Still a permanent fistula may also sometimes yield a normal secretion for a long time (HEIDENHAIN), while the temporary fistula in careless operations may give no secretion or only an abnormal juice.

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[1] See foot-note No. 9, p 123, Chapter VI.

[2] See Minkowski, Arch. f. exp. Path. u. Pharm., Bd. 31, S. 174.

[3] Arch de Physiol., Sér. 5, Tome 7.

[4] Du Bois-Reymond's Arch., 1894.

[5] Leçons de Physiologie, Tome 2, p. 190.

[6] See Bernstein, Arbeiten a. d physiol. Anstalt zu Leipzig, 1869, S. 1.

[7] Pflüger's Arch., Bd. 10 S. [illegible]

In herbivora, such as rabbits, whose digestion is uninterrupted, the secretion of the pancreatic juice is continuous. In carnivora it seems, on the contrary, to be intermittent and dependent on the digestion. During starvation the secretion almost stops, but commences again after partaking of food. Food seems to act in a twofold manner. First, it may, with the more abundant flow of blood during the digestion, which is seen by the red color of the gland, convey a larger quantity of nutritive material to the gland, and thereby cause the secretion of a juice rich in solid nutritive bodies. In another way the food may also, by the irritation which it produces on the mucous coat of the stomach and the duodenum, cause an increased secretion. That the food indeed acts in these two ways follows from the fact that other substances, such as ether, may reflexily act on the mucous membrane of the stomach or intestine, causing a secretion of pancreatic juice, but in starvation a thin fluid is secreted, and after partaking of food a thick fluid is produced. According to the observations of BERNSTEIN, HEIDENHAIN, and others, the secretion increases rapidly after eating, and it reaches its maximum in the course of the first three hours. From this time the secretion diminishes, but may again increase from the 5th–7th hour, when generally large quantities of food pass from the stomach to the intestine. Then it again decreases continuously from the 9th–11th hour, and stops entirely at the 15th–16th hour. In regard to the action of various bodies on the secretion BECKER[1] has found that the introduction of 1–2 gm. sodium chloride or bicarbonate diminishes the quantity of juice secreted by dogs and decreases the proteolytic action of the same, while the introduction of distilled water or, still more, carbonated water increases the secretion. Pilocarpin, according to GOTTLIEB,[1] increases the secretion in rabbits. According to the same investigator the introduction of irritants such as mustard-oil, acids, and alkalies into the duodenum causes reflexly an increased secretion.

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[1] Arch. des Sciences biol. de St. Pétersbourg, Tome 2, No. 3, p. 433.

The statements as to the amount of pancreatic juice secreted in the course of 24 hours are variable and not trustworthy. It seems positively proved that the permanent fistula yields a considerably larger quantity of secretion than the temporary. While KEFERSTEIN and HALLWACHS, and SCHMIDT and KRÖGER, find that the quantity of juice secreted from the first is 45–100 grms. per kilo during 24 hours, BIDDER and SCHMIDT and BIDDER and SKREBITZKY claim that the quantity from the temporary fistula is 2.5–5 grms. per kilo in the same time.[2]

In regard to the *constituents* and *composition* of the pancreatic juice, a distinction must be made between the secretion of a temporary and of a permanent fistula. The secretion flowing from the former is in dogs a clear, colorless, nearly sirupy, odorless fluid of an alkaline reaction which is very rich in proteid, and sometimes containing so large a quantity that it coagulates like white of egg when heated. Besides *proteids* the juice contains also three enzymes—one *diastatic*, one *fat-splitting*, and one which *dissolves proteids*. The last-mentioned has been called *trypsin* by KÜHNE. Besides the above-mentioned bodies the pancreatic juice habitually contains small quantities of *leucin*, *fat*, and *soaps*. As mineral constituents it contains chiefly alkali chlorides, also alkali carbonates, and some phosphoric acid, lime, magnesia, and iron.

The secretion from the permanent fistula always contains less solids, especially proteid and enzymes, than that from a temporary fistula. A long time after the operation it is more fluid, more strongly alkaline, and the property which the juice from the temporary fistula has of dissolving proteids is often absent, or the secretion shows it in only a slight degree. As an example of the different composition of the juice from a temporary and from a permanent fistula we give below the analysis of C. SCHMIDT.[3] The figures represent parts per 1000.

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[1] Arch. f. exp. Path. u. Pharm., Bd. 33.

[2] Cited from Kühne's Lehrbuch., S. 114.

[3] Cited from Maly, Chemie der Verdauungssäfte in Hermann's Handbuch, Bd. 5, Theil 2, S. 189.

	Juice from a Temporary Fistula.		Juice from a Permanent Fistula.		
	a.	*b.*	*a.*	*b.*	*c.*
Water	900.8	884.4	976.8	979.9	984.6
Solids	99.2	115.6	23.2	20.1	15.4
Organic substance	90.4	.....	16.4	12.4	9.2
Ash	8.8	.....	6.8	7.5	6.1

The mineral constituents of the secretion from the temporary fistula consisted chiefly of NaCl, 7.4 p. m.

In the pancreatic juice of rabbits 11–26 p. m. solids have been found, and in that from sheep 14.3–36.9 p. m. In the pancreatic juice of the horse 9–15.5 p. m. solids have been found ; in that of the pigeon, 12–14 p. m.

The human pancreatic juice has been analyzed by HERTER[1] in a case of stoppage of the exit of the juice by the pressure of a cancer. This juice, which could hardly be considered as normal, was clear, alkaline, without odor, and contained the three enzymes. It contained peptone, but no other proteid. The quantity of solids was 24.1 p. m. Of these 6.4 p. m. were soluble in alcohol. It contained 11.5 p. m. peptone (and enzymes) and 6.2 p. m. mineral substances.

ZAWADSKY[2] has analyzed the pancreatic juice of a young woman with a fistula, and found 864.05 p. m. water, 132.51 p. m. organic and 3.44 p. m. inorganic substances. The quantity of protein bodies was 92.05 p. m.

Among the constituents of the pancreatic juice, the three enzymes are the most important.

Amylopsin or pancreatic diastase, which according to KOROWIN[3] and ZWEIFEL[4] is not found in new-born infants and does not appear until more than one month after birth, seems, although not identical with ptyalin, to be nearly related to it. Amylopsin acts very energetically upon boiled starch, especially at + 37° to 40° C., and forms, similar to the action of saliva, besides dextrin, chiefly isomaltose and maltose, with only very little dextrose (MUSCULUS and v. MERING,[4] KÜLZ and VOGEL[5]). The dextrose is probably formed by the action of the invertin[6] existing in the gland and juice.

If the natural pancreatic juice is not to be obtained, then the gland, best after it has been exposed a certain time (24 hours) to the air, may be treated with water or glycerin. This infusion or the glycerin extract diluted with water (when a glycerin has been used which has no reducing action) may be tested directly with starch-paste. It is safer, however, to first precipitate the enzyme from the glycerin extract by alcohol, and wash with this liquid, dry the precipitate over sulphuric acid, and extract with water. The enzyme is dissolved by the water. The detection of sugar may be made in the same manner as in the saliva.

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[1] Zeitschr. f. physiol. Chem., Bd. 4.

[2] Centralbl. f. Physiol., Bd. 5, 1891, S. 179.

[3] See Maly's Jahresber., Bd. 3.

[4] Untersuchungen über den Verdauungsapparat der Neugeborenen. Berlin, 1874.

[5] Zeitschr. f. physiol. Chem., Bd. 2.

[6] See Tebb, Journal of Physiol., Vol. 15, and Abelous, C. R. Soc. de biol., 1891.

Steapsin or **Fat-splitting Enzyme.** The action of the pancreatic juice on fats is twofold. First, the neutral fats are split into fatty acids and glycerin, which is an enzymotic process; and secondly, it has also the property of emulsifying the fats.

The action of the pancreatic juice in splitting the fats may be shown in the following way. Shake olive-oil with caustic soda and ether, siphon off the ether and filter if necessary, then shake the ether repeatedly with water and evaporate at a gentle heat. In this way we obtain a residue of fat free from fatty acids which is neutral, and which dissolves in acid-free alcohol and is not colored red by alkanet tincture. If such fat is mixed with perfectly fresh alkaline pancreatic juice or with a freshly prepared infusion of the fresh gland and treated with a little alkali or with a faintly alkaline glycerin extract of the fresh gland (9 parts glycerin and 1 part 1% soda solution for each gramme of the gland), and some litmus tincture added and the mixture warmed to + 37° C., the alkaline reaction will gradually disappear and an acid one take its place. This acid reaction depends upon the conversion of the neutral fats by the enzyme into glycerin and free fatty acids.

The splitting of the neutral fats may also be shown more exactly by the following method. The mixture of neutral fats (absolutely free from fatty acids) and pancreatic juice or pancreas infusion is digested at the temperature of the body and treated with some soda and repeatedly shaken with fresh quantities of ether until all the unsplit neutral fats are removed. Then it is made acid with sulphuric acid, after which shake the acid liquid with ether, evaporate the ether, and test the residue for fatty acids.

Another simple process for the demonstration of the fat-splitting action of the pancreas glands is the following (CL. BERNARD): A small portion of the perfectly fresh, finely divided gland substance is first soaked in alcohol (of 90%). Then the alcohol is removed as far as possible by pressing between blotting-paper, after which the pieces of gland are covered with an ethereal solution of neutral butter-fat (which may be obtained by shaking milk with caustic soda and ether). After the evaporation of the ether the pieces of gland covered with butter-fat are pressed between two watch-glasses and then gently heated to 37° to 40° C. in this position. After a certain time a marked odor of butyric acid appears.

The action of the pancreatic juice in splitting fats is a process analogous to that of saponification, the neutral fats being decomposed, by the addition of [illegible]ts of water, into fatty acids and glycerin according to the following formula: $C_3H_5.O_3.R_3$ (neutral fat) $+ 3H_2O = C_3H_5.O_3.H_3$ (glycerin) $+ 3(H.O.R)$ (fatty acid). This depends upon a hydrolytic splitting, which was first positively proved by Bernard[1] and Berthelot.[2] The pancreas-enzyme also decomposes other esters just as it does the neutral fats (Nencki,[3] Baas[4]). The pancreas-enzyme which decomposes fats has been less studied than the other pancreas-enzymes, and it has indeed been questioned whether or not the decomposition of the neutral fats in the intestine may not be effected through lower organisms. According to the investigations of Nencki, it seems that the pancreas actually contains an enzyme which decomposes fats. This enzyme, which is still little known, appears to be very sensitive to acids, and it is often absent in acid glands not perfectly fresh. If a watery infusion of the gland prepared cold be treated with calcined magnesia, then the enzyme in question will, according to Danilewski,[5] be retained by the magnesia precipitate.

The fatty acids which are split off by the action of the pancreatic juice combine in the intestine with the alkalies, forming soaps which have a strong emulsifying action on the fats, and thus the pancreatic juice becomes of great importance in the emulsification and the absorption of the fats.

Trypsin. The action of the pancreatic juice in digesting proteids was first observed by Bernard, but first proved by Corvisart.[6] It depends upon a special enzyme called by Kühne trypsin. Strictly speaking, this enzyme does not occur in the gland itself. In the gland, more probably, a zymogen occurs from which the enzyme is split off or formed during secretion, also by the action of water, acids, alcohol, and other substances. According to Albertoni,[7] this zymogen is found in the gland in the last third of the intra-uterine life.

The purest trypsin thus far prepared, isolated by Kühne,[8] is soluble in water, but insoluble in alcohol or glycerin. The less pure

[1] Ann. de chim. et physique (3 sér.), Tome 25.

[2] Jahresber. d. Chem., 1855, S. 733.

[3] Arch. f. exp. Path. u. Pharm., Bd. 20.

[4] Zeitschr. f. physiol. Chem., Bd. 14, S. 416.

[5] Virchow's Arch., Bd. 25.

[6] Gaz. hebdomadaire, 1857, Nos. 15, 16, 19. Cited from Bunge, Lehrbuch, S. 174.

[7] See Maly's Jahresber., Bd. 8, S. 254.

[8] Verh. d. naturh.-med. Vereins zu Heidelberg, (N. F.) Bd. 1, Heft 3.

enzyme, on the contrary, is soluble in glycerin. If the solution of the enzyme in water is heated to the boiling-point with the addition of a little acid, it decomposes into coagulated proteid and peptone (KÜHNE). According to the investigations of BIERNACKI[1] trypsin in 0.25–0.5% soda solution is destroyed in 5 minutes by heating to 50° C. It is destroyed by heating its neutral solution to 45° C. The presence of albumoses or certain ammonium salts in alkaline trypsin solutions have a protective action to a certain extent. Trypsin is destroyed by gastric juice. Like other enzymes, trypsin is characterized by its physiological action. This action consist in dissolving proteids and especially fibrin in alkaline, neutral, or even faintly acid solutions with readiness.

The preparation of pure trypsin has been tried by various experimenters. The purest seems to have been prepared according to the rather complicated method of KÜHNE.[2] In studying the action of trypsin a less pure preparation may often answer, and various methods of preparing such have been proposed, but we cannot describe all of them. For the production of a glycerin extract (HEIDENHAIN[3]) the gland should be rubbed with glass powder or pure quartz-sand, this mass carefully mixed with acetic acid of 1% (1 c. c. to each grm. of gland), then for each part of the gland-mass add 10 parts of glycerin, and filter after about three days. By precipitating the glycerin extract with alcohol and redissolving the precipitate in water, we obtain a solution which has a powerful digestive action. A watery infusion of the gland may be made only after it has been exposed to the air for 24 hours, and 5–10 parts of water for each part by weight of the gland should be used. According to KÜHNE[4] the impure trypsin is allowed to undergo autodigestion in a 0.2% soda solution and in the presence of thymol. After the conversion of the albumoses into peptones the trypsin may be precipitated by ammonium sulphate. An active but impure infusion may be obtained by digesting the finely divided gland for a few days with water containing 5–10 c. c. chloroform per liter (SALKOWSKI[5]).

A very active trypsin may be prepared by extracting the finely divided gland of oxen, free from water and blood, with water containing 0.01–0.05% NH_3. The filtered extract gives a precipitate with acetic acid which has great digestive powers and which can be further purified. (Not published investigations of the AUTHOR.)

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[1] Zeitschr. f. Biologie, Bd. 28.

[2] Verh. d. naturh.-med. Vereins zu Heidelberg, (N. F.) Bd. 1, Heft 3.

[3] Pflüger's Arch., Bd. 10.

[4] Centralbl. f. d. med. Wissensch., 1886, S. 629.

[5] Deutsch. med. Wochenschr., 1888, No. 16.

The *action of trypsin on proteids* is best demonstrated by the use of fibrin. Very considerable quantities of this albuminous body are dissolved by a small amount of trypsin at 37–40° C. It is always necessary to make a control test with fibrin alone, with or without the addition of alkali. Fibrin is dissolved by trypsin without any putrefaction; the liquid has a pleasant odor somewhat like bouillon. To completely exclude putrefaction a little thymol, chloroform, or ether should be added to the liquid. Trypsin digestion differs essentially from pepsin digestion in that the first takes place in neutral or alkaline reaction and not, as is necessary for pepsin digestion, in an acidity of 1–2 p. m. HCl, and further by the fact that the proteids dissolve in trypsin digestion without previously swelling up.

Many circumstances exert a marked influence on the *rapidity of the trypsin digestion.* With an increase in the *quantity of enzyme* present the digestion is hastened at least to a certain point, and the same is also true of an increase in *temperature* at least to about + 40° C., at which temperature the proteid is very rapidly dissolved by the trypsin. The *reaction* is also of the greatest importance. Trypsin acts energetically in neutral, or still better in alkaline, solutions, and best in an alkalinity of 3–4 p. m. Na_2CO_3. Free mineral acids, even in very small quantities, completely prevent the digestion. If the acid is not actually free, but combined with albuminous bodies, then the digestion may take place quickly when the acid combination is not in too great excess (CHITTENDEN and CUMMINS[1]). Organic acids act less disturbingly, and in the presence of 0.2 p. m. lactic acid and the simultaneous presence of bile and common salt the digestion may indeed proceed more quickly than in a faintly alkaline liquid (LINDBERGER[2]). Carbon dioxide, according to SCHIERBECK,[3] has a retarding action in acid solutions, but it accelerates the tryptic digestion in faintly alkaline liquids. *Foreign bodies*, such as borax and potassium cyanide, may promote tryptic digestion, while other bodies, such as salts of mercury, iron, and others (CHITTENDEN and CUMMINS), or salicylic acid in large quantities, may have a preventive action. The *nature of the proteids* is also of importance. Unboiled fibrin is, relatively to most other albuminous bodies, dissolved so very quickly that the digestion test with raw fibrin gives an incorrect idea of the power of trypsin to dissolve coagulated albuminous bodies in general. An *accumulation of products of digestion* tends to hinder the trypsin digestion.

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[1] Studies from the Physiol. Chem. Laboratory of Yale College, New Haven, 1885, Vol. I, p. 100.

[2] See Maly's Jahresber., Bd. 13, S. 280.

[3] Skan. Arch. f. Physiol., Bd. 3.

The Products of the Trypsin Digestion. In the digestion of unboiled fibrin a globulin which coagulates at + 55–60° C. may be obtained as an intermediate product (HERRMANN[1]). Moreover from fibrin, as well as from other albuminous bodies, emanate *albumoses* and *peptones*, *leucin*, *tyrosin*, and *aspartic acid*, a little *lysin*, *lysatinin* (HEDIN[2]), and *ammonia* (HIRSCHLER[3]), and also the so-called *protein chromogen*[4] or *tryptophan*,[5] a substance whose nature is not known, but which gives a reddish-violet product, so-called *proteinochrom*, with chlorine or bromine. When putrefaction has not been entirely prevented numerous other bodies appear which will be spoken of later in connection with the putrefaction process going on in the intestine. In the trypsin digestion, in contrast to the pepsin digestion, pure peptones, not precipitated by ammonium sulphate, are relatively easily and quickly formed. The peptone, according to KÜHNE, consists entirely of antipeptone, and the above-mentioned decomposition products, leucin and the others, are formed by the decomposition of the hemipeptone. We will now consider the decomposition products, leucin and tyrosin, formed in the trypsin digestion of proteids.

Leucin, $C_6H_{13}NO_2$, or AMIDO-CAPROIC ACID, more recently called α-amido-isobutylacetic acid, $(CH_3)_2CH.CH_2.CH(NH_2).COOH$.[6] Leucin is formed not only in the trypsin digestion of proteids, but also from the protein substances by their decomposition on boiling with diluted acids or alkalies, by fusing with alkali hydrates, and by putrefaction. Because of the ease with which leucin and tyrosin are formed in the decomposition of protein substances, it is difficult to positively decide whether these bodies when found in the tissues are constituents of the living body or are only to be considered as decomposition products formed after death.

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[1] Zeitschr. f. physiol. Chem., Bd. 11.

[2] See Drechsel, Du Bois-Reymond's Arch., 1891.

[3] Zeitschr. f. physiol. Chem., Bd. 10, S. 302.

[4] Stadelmann, Zeitschr. f. Biologie, Bd. 26.

[5] Neumeister, *ibid.*, Bd. 26, S. 329.

[6] See Schulze and Likiernik, Zeitschr. f. physiol. Chem., Bd. 17, and Gmelin, *ibid.*, Bd. 18.

Leucin has been found in the pancreas and its secretion, in the spleen, thymus, and lymph-glands, in the thyroid gland, in the salivary glands, in the kidneys, brain, and liver (but mostly in disease). It also occurs in the wool of sheep, in dirt from the skin (inactive epidermis) and between the toes, and its decomposition products have the disagreeable odor of the perspiration of the feet. It is found pathologically in atheromatous cysts, ichthyosis scales, pus, blood, and urine (in diseases of the liver). Leucin also occurs in the vegetable kingdom.

Leucin has been prepared synthetically by HÜFNER[1] from isovaleraldehyde-ammonia and hydrocyanic acid. This leucin is optically inactive. Inactive leucin may also be prepared, as shown by E. SCHULZE, BARBIERI and BOSSHARD,[2] by the cleavage of proteids with baryta at 160° C. or on heating ordinary leucin with baryta-water to the same temperature. The lævorotatory modification may be formed from the inactive leucin by the action of penicillum glaucum. The leucin obtained in the pancreatic digestion of proteids as well as in their cleavage with hydrochloric acid, seems always to be the dextrorotatory variety.[3] COHN[4] has, however, obtained a leucin differing from the ordinary leucin in the tryptic digestion of fibrin. HÜFNER has prepared an isomer of leucin from monobromcaproic acid and ammonia. It is a question whether there exist natural leucins corresponding to normal caproic acid. On oxidation the leucins yield the corresponding oxyacids (leucinic acids). The leucins are decomposed on heating, evolving carbon dioxide, ammonia, and amylamin. On heating with alkalies, as also in putrefaction, it yields valerianic acid and ammonia.

Leucin crystallizes when pure in shining, white, very thin plates, usually forming round knobs or balls, either appearing like hyalin or alternating light or dark concentric layers which consist of radial groups of crystals. Leucin as obtained from the animal fluids and tissues is very easily soluble in water and rather easily in alcohol. Pure leucin is soluble with difficulty; according to certain statements it dissolves in about 29 parts of water at ordinary temperatures or little higher, and according to others in 46 parts. This difference may be due, according to Gmelin,[1] to the fact that the optically active leucin may be variable mixtures of the dextro- and lævorotatory modifications. The inactive leucin is the most insoluble. The specific rotation of the ordinary leucin, dissolved in hydrochloric acid, is $(\alpha)D = +17.5$. Leucin is readily soluble in alkalies and acids. On slowly heating to 170° C. it melts and sublimes in white, woolly flakes which are similar to sublimed zinc oxide. A marked odor of amylamin is generated at the same time.

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[1] Journ. f. prakt. Chem., N. F., Bd. 1.

[2] Zeitschr. f. physiol. Chem., Bdd. 9 and 10.

[3] In regard to contradictory statements see Hoppe-Seyler's Handbuch, 6. Aufl., p. 134.

[4] Zeitschr. f. physiol. Chem., Bd. 20.

The solution of leucin in water is not, as a rule, precipitated by metallic salts. The boiling-hot solution may, however, be precipitated by a boiling-hot solution of copper acetate. If the solution of leucin is boiled with sugar of lead and then ammonia be added to the cooled solution, shining crystalline leaves of leucin-lead oxide separate. When boiled with leucin, copper oxyhydrate is dissolved without reduction.

Leucin is recognized by the appearance of the balls or knobs under the microscope, by its action when heated (sublimation test), and by Scherer's test. This last consists in the leucin yielding a colorless residue when carefully evaporated with nitric acid on platinum-foil, and this residue when warmed with a few drops of caustic soda gives a color varying from a pale yellow to brown (depending on the purity of the leucin), and on further concentrating over the flame it agglomerates into an oily drop which rolls about on the foil.

Tyrosin, $C_9H_{11}NO_3$, or *p*-OXYPHENYL-AMIDOPROPIONIC ACID, $HO.C_6H_4.C_2H_3(NH_2).COOH$, is derived from most protein substances (not gelatin) under the same conditions as leucin, which it habitually accompanies. It is especially found with leucin in large quantities in old cheese (*Τυρός*), from which it derives its name. Tyrosin has not with certainty been found in perfectly fresh organs, with the exception, perhaps, of the spleen and pancreas of cattle. It occurs in the intestine in the digestion of albuminous substances, and it has about the same physiological and pathological importance as leucin.

Tyrosin was prepared by Erlenmeyer and Lipp[2] from p-amido-phenylalanin by the action of nitrous acid. On fusing with caustic alkali it yields p-oxybenzoic acid, acetic acid, and ammonia.

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[1] Zeitschr. f. physiol. Chem., Bd. 18.

[2] Ber. d. deutsch. chem. Gesellsch. zu Berlin, Bd. 15, S. 1544.

By putrefaction it may yield p-hydrocoumaric acid, oxyphenylacetic acid, and p-cresol.

Tyrosin in a very impure state may be in the form of balls similar to leucin. The purified tyrosin, on the contrary, appears as colorless, silky, fine needles which are often grouped into tufts or balls. It is soluble with difficulty in water, being dissolved by 2454 parts water at + 20° C. and 154 parts boiling water, separating, however, as tufts of needles on cooling. It dissolves more easily in the presence of alkalies, ammonia, or a mineral acid. It is difficultly soluble in acetic acid. Crystals of tyrosin separate from an ammoniacal solution on the spontaneous evaporation of the ammonia. The solution of the tyrosin obtained from protein substances by the action of acids has always a faint lævorotatory power. Tyrosin prepared synthetically or by decomposition of proteids by baryta is optically inactive.[1] Tyrosin is not soluble in alcohol or ether. It is identified by its crystalline form and by the following reactions:

PIRIA's *Test.* Tyrosinis dissolved in concentrated sulphuric acid by the aid of heat, by which tyrosin-sulphuric acid is formed; it is allowed to cool, diluted with water, neutralized by $BaCO_3$, and filtered. On the addition of a solution of ferric chloride the filtrate gives a beautiful violet color. This reaction is disturbed by the presence of free mineral acids and by the addition of too much ferric chloride.

HOFMANN's *Test.* If some water is poured on a small quantity of tyrosin in a test-tube and a few drops of MILLON's reagent added and then the mixture boiled for some time, the liquid becomes a beautiful red and then yields a red precipitate. Mercuric nitrate may first be added, then, after this has boiled, nitric acid containing some nitrous acid.

SCHERER's *Test.* If tyrosin is carefully evaporated to dryness with nitric acid on platinum-foil, a beautiful yellow residue (nitrotyrosin nitrate) is obtained, which gives a deep reddish-yellow color with caustic soda. This test is not characteristic, as other bodies give a similar reaction.

Leucin and tyrosin may be prepared in large quantities by boiling albuminous bodies or albuminoids with dilute mineral acids. Ordinarily we boil hoof-shavings (2 parts) with dilute sulphuric acid

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[1] See Mauthner, Wien. Sitzungsber., Bd. 85, and E. Schulze, Zeitschr. f. physiol Chem., Bd. 9.

(5 parts concentrated acid and 13 parts water) for 24 hours. After boiling the solution it is diluted with water and neutralized while still warm with milk of lime and then filtered. The calcium sulphate is repeatedly boiled with water, and the several filtrates are united and concentrated. The lime is precipitated from the concentrated liquid by oxalic acid and the precipitate filtered off, repeatedly boiled with water, all filtrates united and evaporated to crystallization. What first crystallizes consists chiefly of tyrosin with only a little leucin. By concentration a new crystallization may be produced in the mother-liquor, which consists of leucin with some tyrosin. To separate leucin and tyrosin from each other their different solubilities in water may be taken advantage of in preparing them on a large scale, but surer and better results are obtained by the following method of HLASIWETZ and HABERMANN.[1] The crystalline mass is boiled with a large quantity of water and enough ammonia to dissolve it. To this boiling-hot solution enough basic lead acetate is added until the precipitate formed is nearly white; now filter, heat the light yellow filtrate to boiling, neutralize with sulphuric acid, and filter while boiling hot. After cooling, nearly all the tyrosin is precipitated, while the leucin remains in the solution. The tyrosin may be purified by recrystallizing from boiling water or from ammoniacal water. The above-mentioned mother-liquor rich in leucin is treated with H_2S, the filtrate concentrated and boiled with an excess of freshly precipitated copper oxyhydrate. A part of the leucin is precipitated, and the residue remains in the solution and partly crystallizes as a cuprous compound on cooling. The copper is removed from the precipitate and solution by means of H_2S, the filtrate decolorized when necessary with animal charcoal, strongly concentrated and allowed to crystallize. The leucin obtained from the precipitate is quite pure, while that from the solution is somewhat contaminated.

If one is working with small quantities, the crystals, which consist of a mixture of the two bodies, may be dissolved in water and this solution precipitated with basic lead acetate. The filtrate is treated with H_2S, the new filtrate evaporated to dryness, and the residue treated with warm alcohol which dissolves the leucin but not the tyrosin. The remaining tyrosin is purified by recrystallization from ammoniacal alcohol. Leucin may be purified by recrystallization from boiling alcohol or by precipitating it as leucin lead oxide, treating the precipitate suspended in water with H_2S and evaporating the filtered solution to crystallization.

To detect the presence of leucin and tyrosin in animal fluids or tissues the albumin must first be removed by coagulation with the addition of acetic acid and then precipitated by basic lead acetate. The filtrate is treated with H_2S, this filtrate evaporated to a sirup or to dryness, and the two bodies in the residue are separated from each other by boiling alcohol and then purified as above stated.

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[1] Annal. d. Chem. u. Pharm., Bd. 169, S. 160.

Aspartic Acid, $C_4H_7NO_4$, or AMIDO-SUCCINIC ACID, $C_2H_3(NH_2).(COOH)_2$. This acid is obtained in the trypsin digestion of fibrin and gelatin. It may also be obtained by the decomposition of albuminous bodies or albuminoids with acids (see Chapter II). It has also been found in beet-root molasses, and lastly it is very widely diffused in the vegetable kingdom as the amid ASPARAGINE (amido-succinic-acid amid), which seems to be of the greatest importance in the development and formation of the albuminous bodies.

Aspartic acid dissolves in 256 parts water at + 10° C. and in 18.6 parts boiling water, and crystallizes on cooling as rhombic prisms. The acid prepared from protein substances is optically active, and is dextrogyrate in a solution strongly acid with nitric acid, and lævogyrate in a watery solution. It forms with copper oxide a crystalline combination which is soluble in boiling-hot water and nearly insoluble in cold water, and which may be used in the preparation of the pure acid from a mixture with other bodies. In regard to methods of preparation see HLASIWETZ and HABERMANN[1] and E. SCHULZE.[2]

The action of trypsin on other bodies has not been thoroughly studied. An enzyme has been found in the pancreas of the pig and certain herbivora, which is not identical with trypsin and which causes the coagulation of neutral or alkaline *milk* (KÜHNE and ROBERTS[3]). Gelatin is dissolved by the pancreatic juice and is converted into gelatin-peptone. According to KÜHNE and EWALD[4] neither glycocoll nor leucin is formed. The *gelatin-forming substance* of the connective tissues is not directly dissolved by trypsin, but only after it has been treated with acids or soaked in water at + 70° C.

By the action of trypsin on hyalin *cartilage* the cells dissolve, leaving the nucleus. The basis is softened and shows an indistinctly constructed network of collagenous substance (KÜHNE and EWALD). The *elastic substance*, the *structureless membrane*, and the *membrane of the fat-cells* are also dissolved. *Parenchymatous organs*, such as the liver and the muscles, are dissolved all but the nucleus, connective tissue, fat-corpuscles, and the remainder of the nervous tissue. If the muscles are boiled, then the connective tissue is also dissolved. Mucin and certain nucleins are dissolved and split by trypsin solutions. The digestibility of casein pseudonuclein in trypsin solutions has been shown recently by SEBELEIN.[1] POPOFF[2] had previously shown the same for the nuclein from the thymus. GUMLICH[3] and WEINTRAUD[4] have shown that the nucleins are only partly utilized in the intestine.

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[1] L. c.

[2] Zeitschr. f. physiol. Chem., Bd. 9.

[3] See Maly's Jahresber., Bd. 9, S. 224; also Sidney Edkins, Journal of Physiology, Vol. 12, which contains all the literature.

[4] Verh. d. naturh.-med. Vereins zu Heidelberg, (N. F.) Bd. 1.

Trypsin seems to be without action on *chitin* and *horny substance*. *Oxyhæmoglobin* is decomposed by trypsin with the splitting off of hæmatin. *Hæmoglobin*, on the contrary, when the access of oxygen is completely prevented, is not decomposed by trypsin (HOPPE-SEYLER[5]). Trypsin does not act on fats or carbohydrates.

It has already been brought out above that trypsin does not exist ready formed in the gland, but more likely, as HEIDENHAIN[6] has shown, the gland contains a corresponding zymogen. The maximum quantity of such zymogen in the gland occurs 14–16–18 hours after an abundant meal, and the minimum 6–10 hours after. This zymogen is not converted by glycerin into trypsin, but is easily changed by water and acids. A soda solution of 1–1.5%, on the contrary, prevents almost entirely the conversion of the zymogen. If we allow the gland to lie in the air it gradually becomes acid, and this leads to the formation of an enzyme in which the oxygen seems to be active, as is usual in the conversion of the zymogen into trypsin. It is very probable also that the two other enzymes are formed from corresponding zymogens, and this has been shown by LIVERSIDGE[7] to be plausible in the case of the diastatic enzyme.

After a plentiful meal HEIDENHAIN found in dogs in the first stages of digestion, when the secretion of pancreatic juice was most active, that the glandular cells became smaller owing to the consumption of the inner granular zone, while the outer zone at the same time took up new material and became larger. In these stages the quantity of zymogen is smallest. At a later period, 12–20 hours after a meal, the inner zone is re-formed from the outer, and the larger this zone is the larger the quantity of zymogen in the gland seems to be. The zymogen consequently belongs to the inner zone, and the secretion consists therefore, at least in part, in a destruction or dissolution of this zone whereby the substance of the gland itself is changed into the secretion (HEIDENHAIN). This view, however, is in opposition to that of LEWASCHEW,[1] who observed that in animals which have starved and whose pancreas are nearly free from zymogen, the inner granular zone is just as much developed as under normal conditions and containing abundant quantities of zymogen. We are still completely in the dark regarding the nature of the chemical processes which take place in the conversion of the zymogen into the enzyme.

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[1] Zeitschr. f. physiol. Chem., Bd. 20.

[2] *Ibid.*, Bd. 18.

[3] *Ibid.*, Bd. 18.

[4] Verhandl. d. physiol. Gesellsch. zu Berlin, 1895.

[5] Physiol. Chem., S. 267.

[6] Pflüger's Arch., Bd. 10.

[7] Journal of Physiol., Vol. 8.

V. The Chemical Processes in the Intestine.

The action which belongs to each digestive secretion may be essentially changed by mixing with other digestive fluids; and since the digestive fluids which flow into the intestine are mixed with still another fluid, the bile, it will be readily understood that the combined action of all these fluids in the intestine makes the chemical processes going on therein very complicated.

As the acid of the gastric juice acts destructively on ptyalin, this enzyme has no further diastatic action, even after the acid of the gastric juice has been neutralized in the intestine. The bile has, at least in certain animals, a faint diastatic action which in itself can hardly be of any great importance, but which shows that the bile has not a preventive but rather a beneficial influence on the energetic diastatic action of the pancreatic juice. MARTIN and WILLIAMS[2] have observed a beneficial action of the bile on the diastatic action of the pancreas infusion. To this may be added that the organized ferments which occur habitually in the intestine and sometimes in the food have partly a diastatic action and partly produces a lactic-acid and butyric-acid fermentation. The maltose which is formed from the starch seems to be converted into glucose in the intestine. Cane-sugar is inverted in the intestine, but, according to the observations of VOIT and LUSK,[3] milk-sugar is not inverted in the intestine of rabbits. Cellulose, especially the finer and more tender, is undoubtedly partly dissolved in the intestine;

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[1] Pflüger's Arch., Bd. 37.

[2] Proceed. of Roy. Soc., Vols. 45 and 48.

[3] Zeitschr. f. Biologie, Bd. 28, S. 275.

the products formed hereby are not very well known. It has been shown by TAPPENIER that cellulose may undergo fermentation, caused by the action of micro-organisms with the production of marsh-gas, acetic acid, and butyric acid; still we do not know to what extent the cellulose is destroyed in this way.[1]

Bile possesses the power of dissolving fats in so slight a degree that it is scarcely worthy of mention. It is, however, without doubt of greater importance that the bile, as NENCKI[2] and RACHFORD[3] have shown, facilitates the fat-splitting action of the pancreatic juice. This splitting of the fats into fatty acids and glycerin is an important factor in the absorption of the fats. The fatty acids combine with the alkalies of the intestinal and pancreatic juices, producing soaps which are partly absorbed as such and partly exercise a powerful action on the absorption of the fats. There is no doubt that the chief part of the fats in the food is absorbed as a fine emulsion, and the soaps are of great importance in the formation of this emulsion.

If to a soda solution of about 2 p. m. Na_2CO_3 we add pure, perfectly neutral olive-oil in not too large quantity, we obtain, after vigorous shaking, a transient emulsion. If, on the contrary, we add to the same quantity of soda solution an equal amount of commercial olive-oil (which always contains free fatty acids), we need only turn the vessel over for the two liquids to mix and immediately we have a very finely divided and permanent emulsion making the liquid appear like milk. The free fatty acids of the always somewhat rancid commercial oil combine with the alkali to form soaps which act to emulsify the fats (BRÜCKE,[4] GAD[5]). This emulsifying action of the fatty acids split off by the pancreatic juice is undoubtedly assisted by the habitual occurrence of free fatty acids in the food, and also by the splitting off of fatty acids from the neutral fats by the putrefaction in the intestine. These fatty acids must combine with the alkalies in the intestine and form soaps.

[1] On the digestion of cellulose see Henneberg and Stohmann, Zeitschr. f. Biologie, Bd. 21, S. 613; v. Knieriem, *ibid.*, S. 67; Hofmeister, Arch. f. wiss. u. prakt. Thierheilkunde, Bd. 11; Weiske, Zeitschr. f. Biologie, Bd. 22, S. 373; Tappeiner, *ibid.*, Bdd. 20 and 24; and Mallèvre, Pflüger's Arch., Bd. 49.

[2] Arch. f. exp. Path. u. Pharm., Bd. 20.

[3] Journal of Physiol., Vol. 12.

[4] Wien. Sitzungsber., Bd. 61, Abth. 2.

[5] Du Bois-Reymond's Arch., 1878.

This emulsification of fats by means of the action of the pancreatic juice or by soaps formed in other ways can only take place in an alkaline solution. In the contents of the intestine, as long as they are acid, such an emulsion can hardly occur. On the contrary, it undoubtedly occurs at the point where the fat comes in contact with an alkaline secretion under a mucous membrane, or in general where it meets with sufficient alkali to form an emulsion. In the acid contents of the intestine of dogs, which had been kept on food rich in fat, LUDWIG and CASH[1] observed no emulsion. After tying the two pancreas excretory ducts they found a remarkably fine emulsion in the chylous vessels, though the fat in the contents of the intestine was not emulsified. In this case it is possible that the free fatty acid which is hardly ever absent in the fat of the food, and which may be produced also by putrefaction in the intestine, forms soaps with the alkali of the mucous coat of the intestine and produces the emulsion in the chylous vessels. It must not be forgotten that, according to many observations, an emulsion of the fats may be produced by means of proteid, independently of the reaction. In this regard reference should be made to the statement of KÜHNE[2] that the pancreatic juice from a permanent fistula which is poor in proteid has the emulsification property to a less degree than the juice from a temporary fistula which is rich in proteid. KÜHNE has also shown that this emulsification property is not to be ascribed to the alkali, as faintly acid juices also have this property.

CLAUDE BERNARD found long ago in his experiments on rabbits, in which animals the choledochus duct was inosculated to the small intestine above the pancreas passages, that when their food contained a large proportion of fat the chylous vessels of the intestine above the pancreas passages were transparent, but below the same they were milky-white, and from this concluded that the bile alone, without the pancreatic juice, does not emulsify fats. DASTRE[3] tried the reverse experiment in dogs, namely, tying the choledochus duct and producing a biliary fistula, through which the bile would flow into the intestine below the mouth of the pancreatic passages. When the animals were killed after a meal rich in fat, the chylous vessels were first milky-white below the opening of the biliary fistula. DASTRE draws the following conclusion from this: that combined action of the bile and the pancreatic juice is necessary for the absorption of the fats—a deduction which coincides with the above-mentioned observations of NENCKI and RACHFORD. The importance of the bile and the pancreatic juice for the absorption of fats will be discussed in detail later (see Absorption).

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[1] Du Bois-Reymond's Arch., 1880.

[2] Lehrbuch d. physiol. Chem., 1868, S. 123.

[3] Arch. de physiol., (5) Tome 2, p. 315.

Bile completely prevents pepsin digestion in artificial digestion, and it may also retard the swelling up of the proteids. The passage of bile into the stomach during digestion, on the contrary, seems, according to several investigators, especially ODDI[1] and DASTRE,[2] to have no retarding action on stomachic digestion. Bile has no solvent action on proteids in neutral or alkaline reaction, but still it may have an influence on proteid digestion in the intestine. The acid contents of the stomach, containing an abundance of proteids, give with the bile a precipitate of proteids and bile-acids. This precipitate carries a part of the pepsin with it, and for this reason, and also on account of the partial or complete neutralization of the acid of the gastric juice by the alkali of the bile and the pancreatic juice, the pepsin digestion cannot proceed further in the intestine. On the contrary, the bile does not disturb the digestion of proteids by the pancreatic juice in the intestine. The action of these digestive secretions, as above stated, is not disturbed by the bile, especially not by the faintly acid reaction due to organic acids which are habitually found in the upper parts of the intestine. In a dog killed while digestion is going on, the faintly acid, bile-containing matter of the intestine shows regularly a strong digestive action on proteids.

The precipitate formed on the meeting of the acid contents of the stomach with the bile easily redissolves in an excess of bile and also in the NaCl formed in the neutralization of the hydrochloric acid of the gastric juice. This may take place even under faintly acid reaction. Since in man the excretory ducts of the bile and the pancreatic juice open near one another, in consequence of which the acid contents of the stomach are probably immediately in great part neutralized by the bile as soon as it enters, it is doubtful whether a precipitation of proteids by the bile occurs in the intestine.

Besides the previously mentioned processes caused by enzymes, there are others of a different nature going on in the intestine, namely, the fermentation and putrefaction processes caused by micro-organisms. These are less intense in the upper parts of the intestine, but increase in intensity towards the lower part of the same, and decrease in the large intestine because of the absorption of water. Fermentation but not putrefaction processes occur in the small intestine as long as the contents are strongly acid. MACFADYEN, M. NENCKI, and N. SIEBER[1] have investigated a case of human anus præternaturalis, in which the fistula occurred at the lower end of the ileum, and they were able to investigate the contents of the intestine after it had been exposed to the action of the mucous membrane of the entire small intestine. The mass was yellow or yellowish brown, due to bilirubin, had an acid reaction which, calculated as acetic acid, amounted to 1 p. m. The contents were nearly odorless, having an empyreumatic odor recalling that of volatile fatty acids, and only seldom had a putrid odor recalling that of indol. The essential acid present was acetic acid, accompanied with fermentation lactic acid and paralactic acid, volatile fatty acids, succinic acid, and bile acids. Coagulable proteids, peptone, mucin, dextrin, dextrose, and alcohol were present. Leucin and tyrosin could not be detected.

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[1] Centralbl. f. Physiol., 1887, S. 312.

[2] L. c.

According to the above-mentioned investigators, the proteids are only to a very slight extent, if at all, decomposed by the microbes in the small intestine of man. The microbes present in the small intestine preferably decompose the carbohydrates, forming ethyl alcohol and the above-mentioned organic acids. Free hydrochloric acid does not occur in the small intestine, and it is the organic acids that prevent the putrefaction of the proteids in the intestine and also reduce the decomposition of the carbohydrates.

Further investigations of JAKOWSKY[2] lead to the same result, namely, that in man the putrefaction of the proteids does not take place in the small but in the large intestine. This putrefaction of the proteids is not the same as the pancreatic digestion, and these two processes are essentially different because of the products they yield. In the pancreatic digestion of proteids there are formed, as far as we know at present, besides albumoses and peptones, lysin, lysatinin, proteinchromogen, amido-acids, and ammonia. In the putrefaction of the proteids we have, indeed, the same products formed at the beginning, but the decomposition proceeds considerably further and a number of products are developed which have become known through the labors of numerous investigators, NENCKI, BAUMANN, BRIEGER, H. and E. SALKOWSKI, and their pupils. The products which are formed in the putrefaction of proteids are (in addition to *albumoses*, *peptones*, *amido-acids*, and *ammonia*) *indol*, *skatol*, *paracresol*, *phenol*, *phenyl-propionic acid*, and *phenyl-acetic acid*, also *paraoxyphenyl-acetic acid* and *hydroparacumaric acid* (besides paracresol, produced in the putrefaction of tyrosin), *volatile fatty acids*, *carbon dioxide*, *hydrogen*, *marsh-gas*, *methylmercaptan*, and *sulphuretted hydrogen*. In the putrefaction of gelatin neither tyrosin nor indol is formed, while *gycocoll* is produced.

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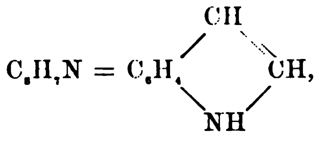
[1] Arch. f. exp. Path. u. Pharm., Bd. 28, S. 311.

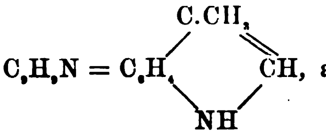
[2] Arch. des sciences biol. de St. Pétersbourg, Tome 1, 1892.

Among these products of decomposition a few are of special interest because of their behavior within the organism and because after their absorption they pass into the urine. A few, such as the oxyacids, pass unchanged into the urine. Others, such as phenol, are directly transformed into ethereal sulphuric acids by synthesis, and are eliminated as such by the urine; on the contrary, others, such as indol and skatol, are only converted into ethereal sulphuric acids after oxidation (for details see Chapter XV). The quantity of these bodies in the urine varies also with the extent of the putrefactive processes in the intestine; at least this is true for the ethereal sulphuric acids. Their quantity increases in the urine with a stronger putrefaction, and the reverse takes place, as BAUMANN[1] has shown by experiments on dogs, when the intestine has been disinfected by calomel, namely, they then disappear from the urine.

Among the above-mentioned putrefactive products in the intestine the two following, indol and skatol. must be carefully discussed.

Indol. 

$$C_8H_7N = C_6H_4 \begin{array}{c} \diagup\, CH \,\diagdown \\ \\ \diagdown\, NH \,\diagup \end{array} CH,$$

and **Skatol**, or METHYL-INDOL, 

$$C_9H_9N = C_6H_4 \begin{array}{c} \diagup\, C.CH_3 \,\diagdown \\ \\ \diagdown\, NH \,\diagup \end{array} CH,$$

are two bodies which stand

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[1] Zeitschr. f. physiol. Chem., Bd. 10.

in close relationship to the indigo substances, and which are formed from the albuminous bodies by their putrefaction, or by fusion with caustic alkali. Hence they occur habitually in the human intestinal canal and, after oxidation into indoxyl and skatoxyl respectively, pass, at least partly, into the urine as the corresponding ethereal sulphuric acids and also as glycuronic acids.

These two bodies have been prepared synthetically in many ways. Both may be obtained from indigo by reducing it with tin and hydrochloric acid and heating this reduction product with zinc-dust (BAEYER[1]). Indol may be formed from skatol by passing it through a red-hot tube. Indol suspended in water is in part oxidized into indigo-blue by ozone (NENCKI[2]).

Indol and skatol crystallize in shining leaves, and their melting-points are + 52° and 95° C. respectively. Indol has a peculiar excrementitious odor, while skatol has an intense fetid odor (skatol obtained from indigo should be odorless). Both bodies are easily volatilized by steam, skatol more easily than indol. They may both be removed from the watery distillate by ether. Skatol is the more insoluble of the two in boiling water. Both are easily soluble in alcohol, and give with picric acid a combination consisting of red crystalline needles. If a mixture of the two picrates be distilled with ammonia, they both pass over without decomposition; while if they are distilled with caustic soda, the indol but not the skatol is decomposed. The watery solution of indol gives with fuming nitric acid a red liquid, and then a red precipitate of nitroso-indol nitrate (NENCKI[3]). It is better to first add two or three drops of nitric acid, and then a 2% solution of potassium nitrite, drop by drop (SALKOWSKI[4]). Skatol does not give this reaction. An alcoholic solution of indol treated with hydrochloric acid colors a pine chip cherry-red. Skatol does not give this reaction. Skatol dissolves in concentrated hydrochloric acid with a violet coloration. On warming skatol with sulphuric acid a beautiful purple-red coloration is obtained (CIAMICIAN and MAGNANINI[5]).

For the detection of indol and skatol in, and their preparation from, excrement and putrefying mixtures, the main points of the usual method are as follows: The mixture is distilled after acidifying with acetic acid; the distillate is then treated with alkali (to combine with any phenol which may be present) and again distilled. From this second distillate the two bodies, after the addition of hydrochloric acid, are precipitated by picric acid. The picrate precipitate is then distilled with ammonia. The two bodies are obtained from the distillate by repeated shaking with ether and evaporation of the several ethereal extracts. The residue, containing indol and skatol, is dissolved in a very small quantity of absolute alcohol and treated with 8–10 vols. of water. Skatol is precipitated, but not the indol. The further treatment necessary for their separation and purification will be found in other works.

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[1] Annal. d. Chem. u. Pharm., Bd. 140, and Supplbd. 7, S. 56; also Ber. d. deutsch. chem. Gesellsch., Bdd. 1 and 3.

[2] Ber. d. deutsch. chem. Gesellsch., Bd. 8, S. 727.

[3] *Ibid.*, Bd. 8, S. 722 and 1517.

[4] Zeitschr. f. physiol. chem., Bd. 8, S. 447.

[5] Ber. d. deutsch. chem. Gesellsch., Bd. 21, S. 1928.

The *gases* which are produced by the decomposition processes are mixed in the intestinal tract with the atmospheric air swallowed with the saliva, and as the gas generated by different foods varies, so the mixture of gases after various foods should have a dissimilar composition. This is found to be true. *Oxygen* is only found in very faint traces in the intestine; this may be accounted for in part by the formation of reducing substances in the fermentation processes which combine with the oxygen, and partly, perhaps chiefly, to a diffusion of the oxygen through the tissues of the walls of the intestine. To show that these processes take place mainly in the stomach the reader is referred to page 278, on the composition of the gases of the stomach. *Nitrogen* is habitually found in the intestine, and it is probably due chiefly to the swallowed air, or perhaps in part, as BUNGE[1] claims, to a diffusion of nitrogen from the tissues of the intestinal walls to the intestine. The *carbon dioxide* originates partly from the contents of the stomach, partly from the putrefaction of the proteids, partly from the lactic-acid and butyric-acid fermentation of carbohydrates, and partly from the setting free of carbon dioxide from the alkali carbonates of the pancreatic and intestinal juices by their neutralization through the hydrochloric acid of the gastric juice and by organic acids formed in the fermentation. *Hydrogen* occurs in largest quantities after a milk diet and in smallest quantities after a purely meat diet. This gas seems to be formed chiefly from the butyric-acid fermentation of carbohydrates, although it may occur in large quantities in the putrefaction of proteids under certain circumstances. There is no doubt that the *methylmercaptan* and *sulphuretted hydrogen* which occurs normally in the intestine originates from the proteids. The

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[1] Lehrbuch d. physiol. u. path. Chem., 1. Aufl., S. 268.

marsh-gas undoubtedly originates in the putrefaction of proteids. As proof of this RUGE[1] found 26.45% marsh-gas in the human intestine after a meat diet. He found a still greater quantity of this gas after a diet consisting of leguminous plants; this coincides with the observation that marsh-gas may be produced by a fermentation of carbohydrates, but especially of cellulose (TAPPEINER[2]). Such an origin of marsh-gas, especially in herbivora, is to be expected. A small part of the marsh-gas and carbon dioxide may also depend on the decomposition of lecithin (HASEBROEK[3]).

Putrefaction in the intestine not only depends upon the composition of the food, but also upon the albuminous secretions and the bile. Among the constituents of bile which are changed or decomposed we have not only the pigments—the bilirubin yields hydrobilirubin and a brown pigment—but also the bile-acids, especially taurocholic acid. Glycocholic acid is more stable, and a part is found unchanged in the excrement of certain animals, while taurocholic acid is so completely decomposed that it is entirely absent in the fæces. In the fœtus, in whose intestinal tract no putrefaction processes occur, we find, on the contrary, undecomposed bile-acids and bile-pigments in the contents of the intestine. The reduction of bilirubin into hydrobilirubin does not, according to MACFADYEN, NENCKI, and SIEBER,[4] take place in man in the small but in the large intestine.

That the secretions rich in proteids are of importance in putrefaction in the intestine follows from the fact that putrefaction may also continue during complete fasting. From the observations of MÜLLER[5] on CETTI it was found that the elimination of indican during starvation rapidly decreased and after the third day of starvation it had entirely disappeared, while the phenol elimination, which at first decreased so that it was nearly minimum, increased again from the fifth day of starvation and on the eighth or ninth day it was three to seven times as much as in man under ordinary circumstances. In dogs, on the contrary, the elimination of indican during starvation is considerable, but the phenol elimination is minimum. Among the secretions which undergo putrefaction in the intestine, the pancreatic juice, which putrefies most readily, takes first place. PISENTI[1] found, in his experiments on dogs, that the elimination of indican by the urine greatly diminished after tying the pancreatic ducts, but that it increased again when the animal was given pancreas peptones or pancreatic juice.

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[1] Wien. Sitzungsber., Bd. 44.

[2] L. c.

[3] Zeitschr. f. physiol Chem., Bd. 12.

[4] Arch. f. exp. Path. u. Pharm., Bd. 28.

[5] Berlin. klin. Wochenschr., 1887, No. 24.

From the foregoing facts we conclude that the products formed by the putrefaction in the intestine are in part the same as those formed in digestion. The putrefaction may be of benefit to the organism so far as the formation of such products as albumoses, peptones, and perhaps also certain amido-acids is concerned. On the contrary, the formation of further splitting products is to be considered as a loss of valuable material, and it is therefore important that putrefaction in the intestine is kept within certain limits. If an animal is killed while digestion in the intestine is going on, the contents of the small intestine give out a peculiar but not putrescent odor. Also the odor of the contents of the large intestine is far less offensive than a putrefying pancreas infusion or a putrefying mixture rich in proteid. From this we may conclude that putrefaction in the intestine is ordinarily not nearly as intense as outside of the organism.

It seems thus to be provided, under physiological conditions, that putrefaction shall not proceed too far, and the factors which here come under consideration are probably of different kinds. Absorption is undoubtedly one of the most important of them, and it has been proved by actual observation that the putrefaction increases, as a rule, as the absorption is checked and fluid masses accumulate in the intestine. The character of the food also has an unmistakable influence, and it seems as if a large quantity of carbohydrates in the food acts against putrefaction (HIRSCHLER[2]).

It has been shown by PÖHL, BIERNACKI, ROVIGHI, WINTERNITZ, and SCHMITZ[3] that milk and kephir have a specially strong preventive action on putrefaction. This action, according to SCHMITZ, is not due to the casein, but chiefly to the lactose and also in part to the lactic acid.

A specially strong preventive action on putrefaction has been ascribed for a long time to the bile. This anti-putrid action is not due to neutral or faintly alkaline bile, which itself easily putrefies, but to the free bile-acids, especially taurocholic acid (MALY and EMICH,[1] LINDBERGER[2]). There is no question that the free bile-acids have a strong preventive action on putrefaction outside of the organism, and it is therefore difficult to deny such an action in the intestine. Notwithstanding this the anti-putrid action of the bile in the intestine is contradicted by certain investigators (VOIT,[3] RÖHMANN[4]).

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[1] See Maly's Jahresber., Bd. 17, S. 277.

[2] Zeitschr. f. physiol. Chem., Bd. 10, S. 306.

[3] *Ibid.*, 17, S. 401, which gives references to the older literature, and Bd. 19. See also Salkowski, Centralbl., f. d. med. Wiss., 1893, S. 467.

Biliary fistulæ have been established so as to study the importance of the bile in digestion (SCHWANN,[5] BLONDLOT,[6] BIDDER and SCHMIDT,[7] and others). As a result it has been observed that with fatty foods an imperfect absorption of fat regularly takes place, and the excrements contain, therefore, an excess of fat and have a light-gray or pale color. The extent of deviation from the normal after the operation is essentially dependent upon the character of the food. If an animal is fed on meat and fat, then the quantity of food must be considerably increased after the operation, otherwise the animal will become very thin, and indeed die with symptoms of starvation. In these cases the excrements have the odor of carrion, and this was considered a proof of the action of the bile in checking putrefaction. The emaciation and the increased want of food depend, naturally, upon the imperfect absorption of the fats, whose high calorific value is reduced and must be replaced by the taking up of larger quantities of other nutritive bodies. If the quantity of proteids and fats be increased, then this last, which can be only very incompletely absorbed, accumulates in the intestine. This accumulation of the fats in the intestine only renders the action of the digestive juices on proteids more difficult, and these last increase the amount of putrefaction. This explains the appearance of fetid fæces, whose pale color is not due to a lack of bile-pigments, but to a surplus of fat (RÖHMANN, VOIT). If the animal is, on the contrary, fed on meat and carbohydrates, it may remain quite normal, and the leading off of the bile does not cause any increased

[1] Monatsheft f. Chem., Bd. 4.

[2] Maly's Jahresber., Bd. 14, S. 334.

[3] Beitr. z. Biologie. Jubiläumsschrift. Stuttgart, 1882.

[4] Pflüger's Arch., Bd. 29.

[5] Miller's Arch. f. Anat. u. Physiol., 1844.

[6] Essai sur les fonctions du foie et de ses annexes. Paris, 1846.

[7] Die Verdauungssäfte und der Stoffwechsel, S. 98.

putrefaction. The carbohydrates may be uninterruptedly absorbed in such large quantities that they replace the fat of the food, and this is the reason why the animal on such a diet does not become emaciated. As with this diet the putrefaction in the intestine is no greater than under normal conditions even though the bile is absent, it would seem that the bile in the intestine exercises no preventive action on putrefaction.

We must remember, however, that the presence of free acids counteracts putrefaction, and further that the carbohydrates yield free acids by acid fermentation within the intestine. It is therefore conceivable that to the carbohydrates, which, according to HIRSCHLER, are capable of checking putrefaction without entering into an acid fermentation, the antiseptic action of the bile is due. It cannot be denied that the bile under ordinary conditions, with a mixed diet deficient in carbohydrates, has a preventive action on the putrefaction in the intestine. LIMBOURG[1] has shown that it acts in an antiseptic sense, so that the destruction of the proteids, giving rise to simpler products less valuable, or perhaps even injurious, in the organism, is checked.

Although the question how the putrefactive processes in the intestine under physiological conditions are kept within certain limits cannot be answered positively, still it may be asserted that the acid reaction of the upper parts of the intestine and the absorption of water in the lower parts are important factors.

That the acid reaction in the intestine has a preventive influence on the putrefactive processes follows from the existing relation between the degree of acidity of the gastric juice and the putrefaction in the intestine. After the investigations and observations of KAST, STADELMANN, WASBUTZKI, BIERNACKI, and MESTER had proven that an increased putrefaction in the intestine occurred when the quantity of hydrochloric acid in the gastric juice was diminished or deficient, SCHMITZ[2] has lately shown in man that on the administration of hydrochloric acid, producing a hyperacidity of the gastric juice, the putrefaction in the intestine may be checked.

Excrements. It is evident that the residue which remains after completed digestion and absorption in the intestine must be different, both qualitatively and quantitatively, according to the variety and quantity of the food. In man the quantity of excrement from a mixed diet is 120–150 grms., with 30–37 grms. solids, per 24 hours, while the quantity from a vegetable diet, according to VOIT,[1] was 333 grms., with 75 grms. solids. With a strictly meat diet the excrements are scanty, pitch-like, and colored nearly black by hæmatin and iron sulphide. The scanty excrements in starvation have a similar appearance. A large quantity of coarse bread yields a great amount of light-colored excrement. If there is a large proportion of fat, it takes a lighter, clayey appearance. The decomposition products of the bile-pigments seem to play only a small part in the normal color of the fæces.

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[1] Zeitschr. f. physiol. Chem., Bd. 13.

[2] *Ibid.*, Bd. 19, S. 401, which includes all the pertinent literature.

The constituents of the fæces are of different kinds. We find in the excrements digestible or absorbable constituents of the food, such as muscle-fibres, connective tissues, lumps of casein, grains of starch, and fat which have not had sufficient time to be completely digested or absorbed in the intestinal tract. In addition the excrements contain indigestible bodies, such as remains of plants, keratin substances, nuclein, and others; also form-elements originating from the mucous coat and the glands; constituents of the different secretions, such as mucin, cholalic acid, dyslysin, and cholesterin; mineral bodies of the food and the secretions; and, lastly, products of putrefaction or of the digestion, such as skatol, indol, volatile fatty acids, lime, and magnesia soaps. Occasionally, also, parasites of different kinds occur; and lastly, the excrements contain micro-organisms, fungi of different kinds, sometimes in such large quantities that the chief mass of the excrements seems to consist of micro-organisms (v. JAKSCH[2]).

That the mucous membrane of the intestine by its secretion and by the abundant quantity of detached epithelium contributes essentially to the formation of excrement follows from the observations first made by L. HERMANN,[3] who separated a loop of intestine, washed it clean and united the two ends, forming a ring, and restored the continuity of the remainder of the intestine. He found in a few days a mass resembling fæces which he called "ring fæces."

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[1] Zeitschr. f. Biologie, Bd. 25, S. 264.

[2] Klinische Diagnostik, 3 Aufl. S. 302.

[3] Pflüger's Arch., Bd. 46. See also Ehrenthal, *ibid.*, Bd. 48; Bernstein, *ibid.*, Bd. 53; Klecki, Centralbl. f. Physiol., 1893, S. 736, and F. Voit, Zeitschr. f. Biologie, Bd. 29.

The reaction of the excrements is very changeable. It is often acid in the inner part, while the outer layers in contact with the mucous coat have an alkaline reaction. In nursing infants it is habitually acid (WEGSCHEIDER[1]). The odor is perhaps chiefly due to skatol, which was first found in the excrements by BRIEGER, and so named by him. Indol and other substances also take part in the production of odor. The color is ordinarily light or dark brown, and depends above all upon the nature of the food. Medicinal bodies may give the fæces an abnormal color. The excrements are colored black by iron and bismuth, yellow by rhubarb, and green by calomel. This last-mentioned color was formerly accounted for by the formation of a little mercury sulphide, but now it is said that calomel checks the putrefaction and the decomposition of the bile-pigments, so that a part of the bile-pigments pass into the fæces as biliverdin. According to LESAGE[2] a green color of the excrements in children is caused partly by biliverdin and partly by a pigment produced from a bacillus. In the yolk-yellow or greenish-yellow excrements of nursing infants we can detect bilirubin. Neither bilirubin nor biliverdin seems to exist in the excrements of mature persons under normal conditions. On the contrary, we find STERCOBILIN (MASIUS and VANLAIR), which, according to certain investigators, is identical with hydrobilirubin (MALY), which is obtained from bilirubin by a reduction process, and urobilin (JAFFÉ)—a view contested by MACMUNN.[3] Bilirubin may occur in pathological cases in the fæces of mature persons. It has been observed in a crystallized state (as hæmatoidin) in the fæces of children as well as of grown persons (UFFELMANN,[4] v. JAKSCH[5]).

The absence of bile (acholic fæces) causes the excrements to have, as above stated, a gray color, due to large quantities of fat; this may, however, be partly attributed to the absence of bile-pigments. In these cases a large quantity of crystals has been observed (GERHARDT, v. JAKSCH) which consist chiefly of magnesia soaps (OESTERLEN) or sodium soaps (STADELMANN[6]). Hemorrhage in the upper parts of the digestive tract yields, when it is not very abundant, a dark-brown excrement, due to hæmatin.

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[1] See Maly's Jahresber., Bd. 6, S. 482.

[2] *Ibid.*, Bd. 18, S. 336.

[3] See Chapter VIII, on the bile, p. 234.

[4] Deutsch. Arch. f. klin. Med., Bd. 24.

[5] Klinische Diagnostik, 4. Aufl., S. 273.

[6] In regard to fat crystals in the fæces see v. Jaksch, l. c., p. 274.

EXCRETIN, so named by MARCET,[1] is a crystalline body occurring in human excrement, but which, according to HOPPE-SEYLER, is perhaps only impure cholesterin. EXCRETOLIC ACID is the name given by MARCET to an oily body with an excrementitious odor.

In consideration of the very variable composition of excrements their quantitative analyses are of little value and therefore will be omitted.

Meconium is a dark brownish-green, pitchy, mostly acid mass without any strong odor. It contains greenish-colored epithelium cells, cell-detritus, numerous fat-globules, and cholesterin plates. The amount of water and solids is respectively 720–800 and 280–200 p. m. Among the solids we find mucin, bile-pigments and bile-acids, cholesterin, fats, soaps, calcium and magnesium phosphates. Sugar and lactic acid, albuminous bodies (?) and peptones, also leucin and tyrosin and the other products of putrefaction occurring in the intestine, are absent. Meconium may contain undecomposed taurocholic acid, bilirubin and biliverdin, but it does not contain any hydrobilirubin, which is considered as proof of the non-existence of putrefactive processes in the digestive tract of the fœtus.

In medico-legal cases it is sometimes necessary to decide whether spots on linen or other substances are caused by meconium. In such cases we have the following conditions: The spot caused by meconium has a brownish-green color and can be easily separated from the material because, on account of the ropy property of the meconium, it is difficult to wet through. When moistened with water it does not develop any special odor, but on warming with dilute sulphuric acid it has a somewhat fetid odor. It forms with water a slimy, greenish-yellow liquid containing brown flakes. The solution gives with an excess of acetic acid an insoluble precipitate of mucin; on boiling it does not coagulate. The filtered, watery extract gives GMELIN'S, but still better HUPPERT'S, reaction for bile-pigments. The liquid precipitated by an excess of milk of lime gives a nearly colorless filtrate, which after concentration gives PETTENKOFER'S reaction.

The contents of the intestine under abnormal conditions are perhaps less the subject of chemical analysis than of an inspection or microscopical investigation. On this account the question as to the properties of the contents of the intestine in different diseases cannot be thoroughly treated here. The question as to the different processes which, so far as they are dependent on secretion and absorption, cause an abnormal consistency, a thinning of the excrements, possesses a certain interest. Such excrements may in part be produced by arrested absorption of liquid from the intestine for some reason or other, and in part caused by an increased secretion or a transudation of liquids into the intestine.

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[1] Annal. de chim. et de phys., Tome 59

A diminished absorption (of water) may be caused by a more active movement of the intestine, which causes their contents to pass quickly, and in this way the action of laxatives is often explained. A diminished absorption may also be due to a decreased activity of the absorbing cells. In absorption, which is generally accepted to-day, the cells of the mucous coat take an active part, and anything which acts disturbingly on the protoplasm of these cells must also exercise an influence on the absorption. This condition with regard to the action of laxatives has been especially noted by HOPPE-SEYLER.[1] According to him, it is also probable that such laxatives, of which only traces are required for absorption, by a direct action on the intestinal epithelium—whether the absorption is made more difficult, or a transudation made possible, or whether the action of these two is simultaneous—cause watery evacuations. According to RÖHMANN,[2] concentrated salt solutions act by a decreased absorption activity.

A thin evacuation may be produced by an increased elimination of fluid into the intestine, and there are many investigators who consider it positively proved that a transudation of liquid into the intestine is caused by the action of saline laxatives.

The character of the intestinal epithelium is undoubtedly an important factor in the production of such a transudation, and when this is caused by the saline laxatives it probably is produced by action on the epithelium. We must admit with HOPPE-SEYLER and other investigators that the most important regulator of the flow of liquid through the intestinal mucous membrane is the intestinal epithelium. It is the epithelium which renders possible the stream of fluid contrary to the laws of osmosis, and which under normal conditions prevents a transudation into the intestine. Bodies which affect the epithelium may therefore cause a transudation, and this is found to be especially abundant after ejection of the intestinal epithelium. The most striking example of this is observed in Asiatic cholera, in which the epithelium is largely expelled and an extraordinarily abundant transudation takes place.

[1] Physiol. Chem., S. 359 and 361.

[2] Pflüger's Arch., Bd. 41.

Appendix.

Intestinal Concrements.

Calculi occur very seldom in human intestine or in the intestine of carnivora, but they are quite common in herbivora. Foreign bodies or undigested residues of food may, when for some reason or other they are retained in the intestine for some time, become incrusted with salts, especially ammonium-magnesium phosphate or magnesium phosphate, and these salts form usually the chief constituent of the concrements. In man they are sometimes oval or round, yellow, yellowish gray, or brownish gray, of variable size, consisting of concentric layers and containing chiefly ammonium-magnesium phosphate, calcium phosphate, besides a small quantity of fat or pigment. The nucleus ordinarily consists of some foreign body, such as the stone of a fruit, a fragment of bone, or something similar. In those countries where bread made from oat-bran is an important food, we often find in the large intestine balls similar to the so-called hair-balls (see below). Such calculi contain calcium and magnesium phosphate (about 70%), oat-bran (15–18%), soaps and fat (about 10%). Concretions which contain very much (about 74%) fat seldom, occur and those consisting of fibrin clots, sinews, or pieces of meat incrusted with phosphates are also rare.

Intestinal calculi often occur in animals, especially in horses fed on bran. These calculi, which attain a very large size, are hard and heavy (as much as 8 kilos) and consist in great part of concentric layers of ammonium-magnesium phosphate. Another variety of concrements which occurs in horses and cattle consists of gray-colored, often very large, but relatively light stones which contain plant residues and earthy phosphates. Stones of a third variety are sometimes cylindrical, sometimes spherical, smooth, shining, brownish on the surface, consisting of matted hairs and plant-fibres, and termed *hair-balls*. The so-called "ÆGAGROPILA," which probably originate from the ANTILOPUS RUPICAPRA, belong to this group, and are generally considered as nothing else than the hair-balls of cattle.

The so-called *oriental bezoar-stone* belongs also to the intestinal concrements, and probably originates from the intestinal tract of the CAPRA ÆGAGRUS and ANTILOPE DORCAS. We may have two varieties of bezoar-stones. One is olive-green, faintly shining, formed of concentric layers. On heating it melts with the development of an aromatic odor. It contains as chief constituent LITHOFELLIC acid, $C_{20}H_{36}O_4$, which is related to cholalic acids, and besides this a bile-acid, LITHOBILIC ACID. The others are nearly blackish brown or dark green, very glossy, consisting of concentric layers, and do not melt on heating. They contain as chief constituent ELLAGIC ACID, a derivative of tannic acid, of the formula $C_{14}H_6O_8$, which gives a deep blue color with an alcoholic solution of ferric chloride. This last-mentioned bezoar-stone originates, to all appearances, from the food of the animal.

Ambergris is generally considered an intestinal concrement of the sperm-whale. Its chief constituent is AMBRAIN, which is a non-nitrogenous substance perhaps related to cholesterin. Ambrain is insoluble in water and is not changed by boiling alkalies. It dissolves in alcohol, ether, and oils.

VI. Absorption.

The problem of digestion consists in part in separating the valuable constituents of the food from the useless constituents and to dissolve or transform these first into forms which are necessary in the processes of absorption. In discussing the absorption processes we must treat of the form into which the different foods are transformed before absorption, of the manner in which this is accomplished, and, lastly, of the forces which act in these processes.

Peptone is the final product of the digestion of albuminous bodies. Now as peptone is a very soluble and a relatively easily diffusible modification of proteids, it is not difficult to admit the deduction that proteids must be changed into peptone in order that it may be readily absorbed. Certain observations of FUNKES[1] on animals confirm this view. He found in an untied intestinal knot of a living animal that the peptone (in the old sense of the word) was absorbed considerably faster than other proteids. There is also no doubt that a part of the proteids is invariably absorbed from the intestinal canal as peptones, or more correctly perhaps as albumoses and peptones. But it has been positively settled by the investigations of BRÜCKE,[2] BAUER and VOIT,[3] EICHHORST,[4] CZERNY and LATSCHENBERGER,[5] that non-peptonized proteids, casein, myosin, and alkali albuminates are absorbed from the intestine—a matter which is of practical importance especially with regard to the nutritive clysters. If the proteids can be absorbed partly as such and partly as peptone or albumoses, then the question arises, how much more can it be absorbed in one form than in the other?

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[1] See Kühne's Lehrb. d. physiol. Chem., S. 145.

[2] Wien. Sitzungsber., Bdd. 37 and 59.

[3] Zeitschr. f. Biologie, Bd. 5.

[4] Pflüger's Arch., Bd. 4.

[5] Virchow's Arch., Bd. 59.

This question cannot be decisively answered. Several investigations have been made on this subject, but it is hardly possible to draw any positive conclusion from them. In feeding experiments on pigs ELLENBERGER and HOFMEISTER[1] found that meat was only slowly digested and the quantity of albumoses and peptones in the intestinal canal was always very small. EWALD and GUMLICH[2] have obtained the same results in regard to the quantity of peptone in normal human stomachs after partaking of meat. Although the albumoses and peptones are rather readily (perhaps more readily than other proteids) absorbed, still it is clear that no positive conclusion can be drawn as to the abundance of peptone-formation from the small quantities of albumoses or peptones found in a certain portion of the intestine. The investigations of SCHMIDT-MÜLHEIM[3] of the contents of the stomach and intestine of dogs who were killed at various times after a meal of boiled meat show that the quantity of peptone in the intestinal canal is considerably larger than the quantity of simply dissolved proteids, and this seems to indicate that in these cases the greatest part of the proteids is absorbed as peptones (or albumoses).

In what way are the albumoses and peptones absorbed, and how are they conveyed to the tissues? LUDWIG and SCHMIDT-MÜLHEIM[4] tied the jugular and humeral arteries and lymphatic vessels of both sides of a dog, completely cutting off the chyle from the blood circulation, as shown later on dissection. They found that the absorption from the intestine hereby was not impaired, and it follows from this that the proteids do not reach the blood through the lymphatic vessels, but through the walls of the intestinal epithelium. The observations of MUNK and ROSENSTEIN[5] on a patient with a lymphatic fistula have led to the same conception. They observed that the quantity of proteid in the chyle did not materially increase after a meal rich in proteids. Neither albumoses nor peptones are found in the chyle after a meal rich in proteids. As the peptones (albumoses included) do not pass in to the lymph, it is to be expected that peptones may be found in the blood during or after digestion. This is not the case. SCHMIDT-MÜLHEIM[1] and HOFMEISTER[2] only found traces of peptone in the serum or blood, and according to NEUMEISTER[3] not even traces exist in the blood.

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[1] Du Bois-Reymond's Arch., 1890.

[2] Berl. klin. Wochenschr., 1890, No. 44.

[3] Du Bois-Reymond's Arch., 1879.

[4] *Ibid.*, 1877, S. 549.

[5] Virchow's Arch., Bd. 123.

What becomes of the peptone absorbed from the intestine? If peptone is introduced into the circulating blood it is quickly eliminated from the blood by means of the urine (PLÓSZ and GYERGYAI,[4] HOFMEISTER,[5] SCHMIDT-MÜLHEIM[6]). The same takes place on the subcutaneous injection of peptone. Normal urine does not contain any peptone, and the absence of this body in the blood after digestion cannot be explained by the statement that an elimination of this peptone takes place through the kidneys. As the peptone introduced in the blood is quickly eliminated through the kidneys, while that formed in the intestine does not pass into the urine, we can perhaps consider that this peptone is retained normally by the liver and is consumed, and only that peptone which finds its way into the circulating blood by evasion from this organ passes into the urine. This supposition, however, is untenable. NEUMEISTER[7] has investigated the portal blood of rabbits in whose stomachs large quantities of albumoses and peptones had been introduced, and found therein only traces of the body in question. He has also shown that when we supply the liver of a dog with the portal-blood peptone (ampho-peptone), this is not retained by the liver, but is eliminated with the urine. SHORE[8] has arrived at similar results in regard to the importance of the liver, and has also shown that the spleen cannot transform peptone. Peptone seems to pass neither into the blood nor the chylous vessels, and the following observation of LUDWIG and SALVIOLI[9] bears out this assumption. These investigators introduced a peptone solution into a double-ligatured, isolated piece of the small intestine, which was kept alive by passing defibrinated blood through it and observed that the peptone disappeared from the intestine, but that the blood passing through did not contain any peptone.

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[1] Du Bois-Reymond's Arch., 1880.

[2] Zeitschr. f. physiol. Chem., Bdd. 5 and 6.

[3] Zeitschr. f. Biologie, Bd. 24, S. 272.

[4] Pflüger's Arch., Bd. 10.

[5] Zeitschr. f. physiol. Chem., Bd. 5.

[6] Du Bois-Reymond's Arch., 1880.

[7] See Neumeister, Sitzungsber. d. phys.-med. Gesellsch. zu Würzburg, 1889, and Zeitschr. f. Biologie, Bd. 24.

[8] Journal of Physiol., Vol. 11.

[9] Du Bois-Reymond's Arch., 1880, Supplement.

All observations indicate that the albumoses and peptones are transformed in some way in the intestine or intestinal wall.

Certain investigators, such as v. OTT,[1] NADINE POPOFF,[2] and JULIA BRINCK[3] are of the opinion that the albumoses and peptones of gastric digestion are transformed into seralbumin before they pass into the walls of the digestive tract. This transformation is brought about by means of the epithelium cells, as also by the living activity of a fungus called by JULIA BRINCK *micrococcus restituens*. No positive proofs have been presented for this view.

The view that the transformation of the albumoses and peptones takes place after they have been taken up by the mucous membrane has better foundation. The above-mentioned experiments of LUDWIG and SALVIOLI confirm this, as do also the observations of HOFMEISTER[4]—according to whom the walls of the stomach and the intestine are the only parts of the body in which peptones occur constantly during digestion—that peptone (at the temperature of the body) after a time disappeared from the excised but apparently still living mucous coat of the stomach. Peptone seems to undergo a change in the mucosa of the digestive canal.

If, then, peptone already disappears in the mucous coat, or at least in the walls of the digestive tract, the question naturally arises, what becomes of the peptone in the mucous membrane? The experiments of MALY,[5] PLÓSZ and GYERGYAI,[6] ADAMKIEWICZ,[7] ZUNTZ,[8] and POLLITZER[9] have established that the albumoses and peptones may be substituted for proteid in the food, and may also be converted into ordinary proteid. We must then assume that peptone is already converted into proteid in the mucous membrane of the digestive canal.

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[1] Du Bois-Reymond's Arch., 1883.

[2] Zeitschr. f. Biologie, Bd. 25.

[3] *Ibid.*, Bd. 25, S. 453.

[4] Zeitschr. f. physiol. Chem., Bd. 6.

[5] Pflüger's Arch., Bd. 9.

[6] L.c.

[7] Die Natur und der Nährwerth des Peptons. Berlin, 1877.

[8] Pflüger's Arch., Bd. 37, S. 313.

[9] *Ibid.*, Bd. 37, S. 301.

According to HOFMEISTER[1] a considerable increase of leucocytes occurs in the adenoid tissues during digestion, an observation which is in close accord with that of POHL,[2] who found that in dogs after an albuminous diet the venous blood of the intestine contains more leucocytes than the arterial blood. According to HOFMEISTER, leucocytes play an important part in the absorption and assimilation of the peptones. They may take up the peptones and be the means of transporting them to the blood, and secondly by their growth, regeneration, and increase may stand in close relation to the transformation and assimilation of the peptones. HEIDENHAIN,[3] who considers that the transformation of peptone into proteid in the mucous membrane is positively settled, does not attribute so great an importance to these last in the absorption of the peptones as HOFMEISTER, chiefly on the ground of comparative estimation of the quantity of absorbed peptones and leucocytes. He considers it most probable that the reconversion of the peptones into proteid takes place in the epithelium layers. This view is further corroborated by the investigations of SHORE.[4]

The extent of the proteid absorption is dependent essentially upon the kind of food introduced, since as a rule the protein substances from an animal source are much more completely absorbed than from a vegetable source. As proof of this we give the following observations: In his experiments on the utilization of certain foods in the intestinal canal of man RUBNER[5] found with an exclusive animal diet on partaking of an average of 738–884 grms. fried meat or 948 grms. eggs per day that the nitrogen deficit with the excrement was only 2.5–2.8% of the total introduced nitrogen. With exclusive milk diet the results were somewhat unfavorable, since after partaking of 4100 grms. milk the nitrogen deficit rose indeed to 12%. The conditions are quite different with vegetable food, as shown by the experiments of MEYER,[6] RUBNER,[7] HULTGREN and LANDERGREN,[1] who made experiments with various kinds of rye bread and found that the loss of nitrogen through the fæces amounted to 22–48%. Experiments with other vegetable foods, and also the investigations of SCHUSTER,[2] CRAMER,[3] MEINERT,[4] MORI,[5] and others on the utilization of foods with mixed diets, have led to similar results. All through we see that the loss of nitrogen by the excrement increases with an abundant amount of vegetable food in the diet.

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[1] Arch. f. exp. Path. u. Pharm., Bdd. 19, 20, and 22.

[2] *Ibid.*, Bd. 25.

[3] Pflüger's Arch., Bd. 43.

[4] L. c.

[5] Zeitschr. f. Biologie, Bd. 15.

[6] *Ibid.*, Bd. 7.

[7] *Ibid.*, Bd. 15.

The reason for this is manifold. The often large quantity of cellulose present in vegetable foods impedes the absorption of proteids. The stronger irritation produced by the vegetable food itself or by the organic acids formed in the fermentation in the intestinal canal causes a stronger peristalsis which drives the contents of the intestine quicker than otherwise along the intestinal canal. Another and most important reason is the fact that a part of the vegetable protein substances seems to be indigestible.

In speaking of the functions of the stomach we stated that after the removal or excision of this organ an abundant digestion and absorption of proteids may take place. It is therefore of interest to learn how the digestion and absorption of proteids go on after the extirpation of the second proteid-digesting organ, the pancreas. In this regard MINKOWSKI and ABELMANN[6] found after the total extirpation of the pancreas of dogs that the utilization of the proteids was on an average 44%, and after partial extirpation 54%. SANDMEYER[7] found in dogs after the extirpation of $\frac{8}{9}$ or $\frac{4}{5}$ of the gland, and leaving pieces not in connection with the intestine, that the utilization of the proteids amounted to 62–70%. All three investigators found that on the addition of raw ox-pancreas to the food the utilization of the proteids was essentially increased, and on the addition of sufficient finely chopped pancreas SANDMEYER observed even a proteid absorption which did not differ much from that of a normal dog. It seems that the destructive action of the gastric juice on the trypsin does not assert itself under these circumstances, or only to a slight extent.

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[1] Nord. med. Arkiv., Bd. 21, No. 8.

[2] See Voit, Untersuch. der Kost, etc., S. 142.

[3] Zeitschr. f. physiol. Chem., Bd. 6.

[4] Ueber Massenernährung. Berlin, 1885.

[5] Kellner and Mori, Zeitschr. f. Biologie, Bd. 25.

[6] Ueber die Ausnutzung der Nahrungsstoffe nach Pankreasexstirpation, etc. Inaug. Diss. Dorpat, 1890. Cited from Maly's Jahresber., Bd. 20.

Zeitschr. f. Biologie, Bd. 31.

The carbohydrates are, it seems, chiefly absorbed as monosaccharides. Glucose, lævulose, and galactose are probably absorbed as such. The two disaccharides, cane-sugar and maltose, ordinarily undergo an inversion in the intestinal tract and are converted into glucose and lævulose. Lactose, according to VOIT and LUSK,[1] is not inverted and is absorbed as such except what undergoes lactic-acid fermentation. The polysaccharides are also finally converted into monosaccharides, although in certain cases an absorption of dextrin may take place. According to the observations of OTTO[2] and v. MERING[3] the portal blood contains besides dextrose a dextrin-like carbohydrate after a carbohydrate diet. A part of the carbohydrates is destroyed by fermentation in the intestine, with the formation of lactic and acetic acids.

The different varieties of sugars are absorbed with varying degrees of rapidity, but as a general thing they are absorbed very quickly. With experiments on dogs ALBERTONI[4] found on introducing 100 grms. of the sugar that during the first hour 60 grms. dextrose were absorbed, maltose and cane-sugar 70–80, and lactose only 20–40 grms. He finds that lactose is relatively more readily absorbed from dilute solutions than from concentrated ones.

On the introduction of starch even in very considerable quantities into the intestinal tract no dextrose passes into the urine, which probably depends in this case upon the absorption and assimilation and the slow saccharification taking place at the same pace. If, on the contrary, large quantities are introduced at one time, then an elimination of sugar by the urine takes place, and this elimination of sugar is called *alimentary glycosuria*. In these cases the assimilation of the sugar and the absorption do not take place at the same pace, hence the liver and the remaining organs do not have the necessary time to fix and utilize the sugar. This glycosuria may also in part be due to the fact that the introduction of considerable quantities of sugar forces the sugar in absorption not only in the ordinary way through the blood-vessels to the liver (see below), but also in part by passing into the blood circulation through the lymphatic vessels, evading the liver.

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[1] Zeitschr. f. Biologie, Bd. 28.

[2] Christiania Vidensk. Selskabs Forh., 1886, No. 11, and Maly's Jahresber., Bd. 17.

[3] Du Bois-Reymond's Arch., 1877.

[4] Manière de se comporter des sucres, etc. Arch. ital. de Biol., Tome 15.

That quantity of sugar to which we must raise the sugar partaken of to produce an alimentary glycosuria gives, according to HOFMEISTER,[1] the *assimilation limit* for that same sugar. This limit is different for various kinds of sugar; and it also varies for the same sugar not only in different animals, but also for different members of the same kind, as also for the same individual under different circumstances. In general we can say that in regard to the ordinary varieties of sugar, such as dextrose, lævulose, cane-sugar, maltose, and lactose, the assimilation limit is highest for dextrose and lowest for lactose. We must admit that with an overabundant quantity of sugars in the intestinal tract the disaccharides do not have sufficient time for their complete inversion; hence it is not remarkable that disaccharides have been found in the urine in cases of alimentary glycosuria.[2]

From the investigations of LUDWIG and v. MERING[3] and others we learn in regard to the way in which the sugars pass into the blood-stream, namely, that they as well as bodies soluble in water do not ordinarily pass over into the chylous vessels in measurable quantities, but are in greatest part taken up by the blood in the capillaries of the villi and in this way pass into the mass of the blood. These investigations have been confirmed by observations of I. MUNK and ROSENSTEIN[4] on human beings.

The reason why the sugar and other soluble bodies do not pass over into the chylous vessels in appreciable quantity is, according to HEIDENHAIN,[5] to be found in the anatomical conditions, in the arrangement of the capillaries close under the layer of epithelium. Ordinarily these capillaries find the necessary time for the taking up of the water and the solids dissolved in it. But when a large quantity of liquid, such as a sugar solution, is introduced into the intestine at once, this is not possible, and in these cases a part of the dissolved bodies passes into the chylous vessels and the thoracic duct (GINSBERG[6] and RÖHMANN[7]).

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[1] Arch. f. exp. Path. u. Pharm., Bdd. 25 and 26.

[2] For the literature in regard to the passage of various kinds of sugars into the urine see C. Voit, Ueber die Glykogenbildung, Zeitschr. f. Biologie, Bd. 28.

[3] Du Bois-Reymond's Arch., 1877.

[4] L. c.

[5] Pflüger's Arch., Bd. 43, Suppl.

[6] *Ibid.*, Bd. 44.

[7] *Ibid.*, Bd. 41.

The introduction of larger quantities of sugar into the intestine at one time can readily cause a disturbance with diarrhœal evacuations of the intestine. If the carbohydrate is introduced in the form of starch, then very large quantities may be absorbed without causing any disturbance and the absorption may be very complete. RUBNER[1] found the following: On partaking 508–670 grms. carbohydrate as wheat bread per day the part not absorbed amounted to only 0.8–2.6%. For peas, where 357–588 grms. were eaten, the loss was 3.6–7%, and for potatoes (718 grms.) 7.6%. CONSTANTINIDI[2] found on partaking 367–380 grms. carbohydrates, chiefly as potatoes, a loss of only 0.4–0.7%. In the experiments of RUBNER,[3] as also of HULTGREN and LANDERGREN,[4] with rye bread the utilization of carbohydrates was less complete, although the loss in a few cases rose even to 10.4–10.9%. It at least follows from the experiments made thus far that man can absorb more than 500 grms. carbohydrates per diem without difficulty.

We generally consider the pancreas as the most important organ in the digestion and absorption of amylaceous bodies, and it is a question how these bodies are absorbed after the extirpation of the pancreas. MINKOWSKI and ABELMANN[5] found that in dogs after total extirpation of the pancreas only 57–71% of the amylaceous bodies were absorbed. In the experiments of the brothers CAVAZZANNI[6] only 47% of the starch introduced was used by the animal with the pancreas removed.

Emulsification seems to be of the greatest importance in the absorption of fats. The fats may be absorbed in part as soaps, but the quantity absorbed in this form is very small as compared to that which is absorbed as an emulsion. The emulsion is undoubtedly the most important form in which fats are absorbed, and the neutral fats as well as the free fatty acids, when they occur in large quantities in the intestine, form an emulsion. The fatty acids are not absorbed as such or as soaps. The investigations of I. MUNK,[7] and later confirmed by others,[8] have shown that the fatty acids undergo in great part a synthesis into neutral fats in the walls of the intestine or, according to WALTHER,[1] in the intestine, and carried as such by the stream of chyle into the blood.

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[1] L. c. and Zeitschr. f. Biologie, Bd. 19.

[2] Zeitschr. f. Biologie, Bd. 23.

[3] *Ibid.*, Bd. 15.

[4] Nord. med. Arkiv., Bd. 21.

[5] L. c. See Maly's Jahresber., Bd. 20.

[6] Centralbl. f. Physiol., Bd. 7.

[7] Virchow's Arch., Bd. 80.

[8] See v. Walther, Du Bois-Reymond's Arch., 1890, and Minkowski, Arch. f exp. Path. u. Pharm., Bd. 21, S. 373.

Through numerous investigations we also know that among all the nutritive bodies the fats are the only substances that under ordinary conditions pass into the blood through the lymphatic vessels and the thoracic duct. It does not follow from this that all or the greater part of the fat takes this course, and according to the experiments of v. WALTHER and O. FRANK[2] the reverse is true, namely, only a very small part of the fat, or at least of the fatty acids partaken of, passes into the chylous vessels. On feeding dogs with fatty acids WALTHER found that in the course of several hours only very few grammes of fat were carried away with the lymph current, although the intestine had absorbed 40–50 grms. fat. FRANK has reached a similar conclusion, and indeed found that by the excision of the thoracic duct an absorption of fatty acids took place to a considerable extent. These observations do not seem to be applicable to the absorption of neutral fats or of the absorption in man under normal conditions. MUNK and ROSENSTEIN in their investigations on a girl with lymph fistula found 60% of the fat partaken of in the chyle, and of the total quantity of fat in the chyle only 4–5% existed as soaps. On feeding with a foreign fatty acid, such as erucic acid, they found 37% of the introduced body as neutral fat in the chyle.

The completeness with which fats are absorbed depends, under normal conditions, essentially upon the kind of fat. In this regard we know, especially from the investigations of MUNK[3] and ARNSCHINK,[4] that the varieties of fat with high melting-points, such as mutton tallow and especially stearin, are not so completely absorbed as the fats with low melting-points, such as hog- and goose-fat, olive-oil, etc. The kind of fat also has an influence upon the rapidity of absorption, as MUNK and ROSENSTEIN found that solid mutton-fat was absorbed more slowly than fluid lipanin. The extent of absorption in the intestinal tract is under physiological conditions very considerable. In a case of a dog investigated by VOIT[5] he found that out of 350 grms. of fat (butter) partaken, 346 grms. were absorbed in the intestinal canal, and according to the investigations of RUBNER[1] the human intestine can absorb over 300 grms. fat per diem. The fats are, according to RUBNER, much more completely absorbed when free, in the form of butter or lard, than when enclosed in the cell-membranes, as in bacon.

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[1] Walther, l. c.

[2] Du Bois-Reymond's Arch., 1892.

[3] Virchow's Arch., Bdd. 80 and 85.

[4] Zeitschr. f. Biologie, Bd. 26.

[5] *Ibid.*, Bd. 9.

The bile as well as the pancreas is of the greatest importance in the absorption of fats.

Through numerous observations of many investigators, such as BIDDER and SCHMIDT,[2] VOIT,[3] RÖHMANN,[4] FR. MÜLLER,[5] I. MUNK,[6] and others, it has been shown that the exclusion of the bile from the intestinal tract diminishes the absorption of fat to such an extent that only ¼ to about ½ of the quantity of fat ordinarily absorbed undergoes absorption. In icterus with entire exclusion of the bile a considerable decrease in the absorption of fat is noticed. As under normal conditions, so also in the absence of bile in the intestine the more readily melting parts of the fats are more completely absorbed than those which have a high melting-point. I. MUNK found in his experiments with lard and mutton tallow on dogs that the absorption of the high melting tallow was reduced twice as much as the lard on the exclusion of the bile from the intestine.

We also learn from the investigations of RÖHMANN and I. MUNK that in the absence of bile the relationship between fatty acids and neutral fats is changed, namely, about 80–90% of the fat existing in the fæces consists of fatty acid, while under normal conditions the fæces contain 1 part neutral fat to about 2–2½ parts free fatty acids. We cannot positively state how this relatively increased quantity of fatty acids in the fat of the fæces is produced on the exclusion of the bile from the intestine. According to the investigations of MUNK it does not in the least depend upon the fact that the fatty acids are less readily absorbed than the neutral fats, for just the reverse is the case.

There is no doubt that the bile is of great importance in the absorption of fats. Still there is also no doubt that rather considerable quantities of fat may be absorbed from the intestine in the absence of bile. What relation does the pancreas bear to this question?

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[1] Zeitschr. f. Biologie, Bd. 15.

[2] Die Verdauungssäfte und der Stoffwechsel, S. 223.

[3] Beiträge z. Biologie, Jubiläumsschrift für v. Bischoff. Stuttgart, 1882.

[4] Pflüger's Arch., Bd. 29.

[5] Sitzungsber. d. physik.-med. Gesellsch. zu Würzburg, 1885.

[6] Virchow's Arch., Bd. 122.

According to BERNARD the presence of pancreatic juice in the intestine is necessary in the absorption of fats. This view has found support in the investigations of MINKOWSKI and ABELMANN[1] on the absorption of fats after the extirpation of the pancreas in dogs. These investigators found that the fat introduced in the food was not absorbed at all after the complete extirpation of the pancreas. Milk was an exception, and a greater or smaller part (28–53%) of its fat was absorbed.

It is difficult to state anything positive about the significance of these observations, since there are other investigations which have led to different results. SANDMEYER[2] found in his experiments on dogs that the utilization of the non-emulsified fat was very variable. Sometimes no fat was absorbed, while at other times in the same animal 30 or even 78% of the administered fat was absorbed. In a series of experiments administering emulsified fat in the form of milk about 42% was absorbed. TEICHMANN[3] has also found that after ligaturing the pancreatic duct in rabbits the absorption of fat was not noticeably disturbed, and FR. MÜLLER[4] had occasion to observe in a patient with pancreatic fistula that in human beings a considerable absorption of fat may take place in the intestine without pancreatic juice.

The question as to the importance of the pancreatic juice in the absorption of fats is still somewhat disputed. It is certain at least that the pancreatic juice is of very great importance for the absorption of fats, and it is also certain that the absorption of fats is most considerable in the simultaneous presence of bile and pancreatic juice in the intestine. We can give no explanation for this last fact. The common acceptance is that to form an emulsion of the fats a previous splitting is necessary, and this is produced by the pancreatic juice, accelerated by the bile. Many doubts have been raised against this statement, and to what has been said already (page 310) we must add the following: In the experiments of MINKOWSKI and ABELMANN the masses of fat eliminated by the fæces were in great part split even in the absence of the pancreas, and according to the investigations of HÉDON and WILLE[1] an abundant splitting of the fats may take place in the intestine even in the absence of the bile as well as of the pancreatic juice. The extent of action of microbes and other unknown factors in this splitting has not yet been determined.

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[1] Ueber die Ausnutzung der Nahrungsstoffe nach Pankreasexstirpation, etc. Inaug. Diss. Dorpat, 1890.

[2] Zeitschr. f Biologie, Bd. 31.

[3] Mikroskop. Beitr. z. Lehre von der Fettresorption. Diss. Breslau, 1891. Cited from Neumeister, Lehrb. d. physiol. Chem. Jena, 1897. S. 336.

[4] Cited from Neumeister, Lehrb. d. physiol. Chem. Jena, 1897. S. 337.

From these experiments we cannot draw any positive conclusion as to the importance of the splitting of the fat for emulsification under normal conditions, because on the exclusion of the pancreatic juice from the intestine the secretion of alkali carbonates, which are important in the emulsification of the fats as well as for the normal processes in the intestine, suffers essentially in quantity. In the experiments of MINKOWSKI and ABELMANN the ethereal extract of the fat masses of the fæces consisted of 80% fatty acids, which were chiefly free and only combined with alkali to a slight extent.

V. HARLEY[2] has made experiments on the absorption of fats (milk) in dogs with extirpated pancreas. The passage of the fats from the stomach to the intestine in these dogs was retarded, and HARLEY found not only as much fat in the intestinal tract as was introduced, but also a little which was derived from the secretions and excretions of the intestine. This experiment gave entirely different results from ABELMANN's experiment, and HARLEY explains this by the fact that in ABELMANN's experiment the action of intestinal bacteria was not excluded or reduced to a minimum, as in his.

The fact that milk is the only form in which fat can be absorbed in dogs in the absence of pancreatic juice (MINKOWSKI) may, according to him, be explained in the fact that this fat emulsion is permanent in acid as well as in neutral or alkaline reaction. From these observations, and from the confirming observations of SANDMEYER that a considerable absorption of other fats may take place in dogs with extirpated pancreas when with the fat food we add finely chopped ox-pancreas, MINKOWSKI suggests that the proteids are of the greatest importance in the emulsification of fats. This view is in accordance with the older statements of BERNARD and KÜHNE,[3] but has not been the subject of thorough research.

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[1] See Maly's Jahresber., Bd. 22, S. 38.

[2] Journal of Physiol., Vol. 18.

[3] See page 311.

The soluble salts are also absorbed with the water. The proteids and peptone which can dissolve a considerable quantity of salts, such as earthy phosphates which are otherwise insoluble in alkaline water, are of great importance in the absorption of such salts.

Water, according to the experiments of v. MERING,[1] as also of GLEY and RONDEAU,[2] on dogs is not absorbed to any appreciable amount in the stomach. Alcohol, on the contrary, is absorbed to a great extent in the stomach. The extent of the absorption of dissolved bodies seems to be dependent upon the concentration of the solution, and according to BRANDL[3] the difference between the absorption in the stomach and intestine consists in that the absorption in the first organ takes place better in greater concentration and in the second in less concentration. Thus, for example, a solution of cane- or grape-sugar is most completely absorbed by the intestine in a concentration of 0.5%. With increasing concentration the absorption diminishes, and in a concentration of 5% a disturbance takes place. In the stomach an appreciable absorption first occurs with a concentration of 5%, and then increases to about 20%. The presence of other bodies which cause an irritation on the mucous membrane seems to be valuable for absorption, and according to BRANDL chloral hydrate, sugar, and potassium-iodide solutions are better absorbed in the presence of alcohol than from pure watery solution.

The soluble constituents of the digestive secretions may, like other dissolved bodies, be absorbed, as is demonstrated by the passage of peptone into urine; the enzymes may also be absorbed. The occurrence of urobilin in urine attests the absorption of the bile-constituents under physiological conditions notwithstanding the question as to the occurrence of very small traces of bile-acids in the urine is disputed. The absorption of bile-acids by the intestine seems to be positively proven by other observations. TAPPEINER[4] introduced a solution of bile-salts of a known concentration into an intestinal knot, and after a time investigated the contents. He found that in the jejunum and the ileum, but not in the duodenum, an absorption of bile-acids took place, and further that of the two bile-acids only the glycocholic acid was absorbed in the jejunum. Further, SCHIFF[1] long ago expressed the opinion that bile undergoes an intermediate circulation, in such wise that it is absorbed from the intestine, then carried to the liver by the blood, and lastly eliminated from the blood by this organ. Although this view has met with some opposition, still its correctness seems to be established by the researches of various investigators, and more recently by PREVOST and BINET,[2] as also and specially by STADELMANN and his pupils.[3] After the introduction of foreign bile into the intestine of an animal the foreign bile-acids appear again in the secreted bile.

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[1] Centralbl. f. Physiol., Bd. 7, S. 533.

[2] C. R. Soc. de Biol., 1893.

[3] Zeitschr. f. Biologie, Bd. 29. This contains all the older literature relating to this question.

[4] Wien. Sitzungsber., Bd. 77.

Little is known concerning the forces taking part in absorption. Osmosis and filtration were formerly considered as the most important factors. But as in regard to the peptones, whose formation in the digestion was considered as taking place especially in the interest of a facilitated osmosis and filtration, but whose conditions, have been found quite different and much more complicated, so in the absorption theory there is a still greater contrast between former and present views, the latter inclining to the theory that absorption is a process connected with the vital properties of the cells (HOPPE-SEYLER[4]). Investigations in this direction have been made by HEIDENHAIN[5] and his pupils RÖHMANN[6], and GUMILEWSKY[7]; and these investigations have shown that the cells take an active part in the absorption, and that this action is independent of the processes caused by an unequal diffusibility of the different bodies. For example, in a solution which contains equal quantities of grape-sugar and sodium sulphate the sugar will be almost completely absorbed in a certain time, while the salt, which has the greater diffusibility, still remains in considerable amounts in the intestine. According to the latest investigations of HEIDENHAIN[8] on the absorption of blood-serum and common-salt solutions from the intestine of dogs, no doubt can now exist that the cells have a special physiological force besides which under certain circumstances osmosis may operate, but under other circumstances an absorption may take place with the entire exclusion of osmosis. It is also known that certain pigments are absorbed and others not, and the cells seem to have the property of discriminating between the different substances. The absorption of dissolved bodies seems to be connected with a specific activity of the living cell, the living protoplasm.

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[1] Pflüger's Arch., Bd. 3.

[2] Compt. rend., Tome 106.

[3] See reference, page 224.

[4] Physiol. Chem., S. 348.

[5] Pflüger's Arch., Bdd. 43 and 56.

[6] *Ibid.*, Bd. 41.

[7] *Ibid.*, Bd. 39.

[8] *Ibid.*, Bd. 56.

In the absorption of bodies not dissolved, of the emulsified fats, forces take part which are not known. That the bile performs the most important part in the absorption of fats is very generally admitted, but how the bile acts in this process is not yet determined. V. WISTINGHAUSEN[1] has found that fat rises higher in a capillary tube moistened with bile than when moistened with water, and further that fluid fat filters more easily through a dead membrane dipped in bile than when dipped in water. From these observations, whose correctness has lately been disputed by GAD and GRÖPER,[2] the inference has been drawn that bile facilitates the capillary attraction and thereby accelerates the absorption of the fats. The epithelium layer of the intestinal mucous membrane cannot be compared with a dead membrane soaked in water, and it is therefore doubtful if the above-mentioned action of bile can have any influence on the absorption of fats in the intestine. That the absorption of fats is caused by the lymphoid migratory cells (ZAWARYKIN,[3] SCHÄFER[4]) is disputed by GRUENHAGEN[5] and HEIDENHAIN.[6] According to them, the fat takes its way chiefly through the epithelium cells. How these last act is, like the nature of their action in absorption, enveloped in darkness.

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[1] See the translation of Wistinghausen's dissertation by Steiner in Du Bois-Reymond's Arch., 1873.

[2] Du Bois-Reymond's Arch., 1889.

[3] Pflüger's Arch., Bd. 31.

[4] *Ibid.*, Bd. 33.

[5] Arch. f. mikroskop. Anat., Bd. 29.

[6] Pflüger's Arch., Bd. 43.

CHAPTER X.

TISSUES OF THE CONNECTIVE SUBSTANCE.

I. The Connective Tissues.

THE form-elements of the typical connective tissues are cells of various kinds, of a not very well known chemical composition, and gelatin-yielding fibrils, which like the cells are imbedded in an interstitial or intracellular substance. The fibrils consist of *collagen*. The interstitial substance contains chiefly *mucin* besides *serglobulin* and *seralbumin*, which occur in the parenchymatous fluid (LOEBISCH[1]).

The connective tissue also often contains fibres or formations consisting of elastin, sometimes in such great quantities that the connective tissue is transformed into elastic tissue. According to MALL[2] a third variety of fibres, the reticular fibres, also occur, and these according to SIEGFRIED consist of RETICULIN.

If finely divided tendons are extracted in cold water, the albuminous bodies soluble in the nutritive fluid in addition to a little mucin are dissolved. If the residue is extracted with half-saturated lime-water, then the mucin is dissolved (ROLLETT,[3] LOEBISCH) and may be precipitated from the filtered extract by saturating with acetic acid. The digested residue contains the fibrils of the connective tissue together with the cells and the elastic substance.

The fibrils of the connective tissue are elastic and swell slightly in water, somewhat more in dilute alkalies or in acetic acid. On the other hand, they shrink by the action of certain metallic salts, such as ferrous sulphate or mercuric chloride, and tannic acid, which forms an insoluble combination with the collagen. Among these combinations, which prevent putrefaction of the collagen, that with tannic acid has been found of the greatest technical importance in the preparation of leather. In regard to tendon mucin see page 45, and in regard to collagen, gelatin, elastin, and reticulin, pages 51–56.

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[1] Zeitschr. f. physiol. Chem., Bd. 10.

[2] Kgl. Sächs. Gesellsch. d. Wissensch., 1891, Bd. 17, Math.-phys. Klasse.

[3] Wien. Sitzungsber., Bd. 39.

The tissues described under the names *mucous* or *gelatinous tissues* are characterized more by their physical than their chemical properties and have been but little studied. So much, however, is known, that the mucous or gelatinous tissues contain, at least in certain cases, as in the acalephæ, no mucin.

The umbilical cord is the most accessible material for the investigation of the chemical constituents of the gelatinous tissues. The mucin occurring therein has been described on page 45. C. TH. MÖRNER[1] has found a *mucoid* in the vitreous humor which contains 12.27% nitrogen and 1.19% sulphur.

Young connective tissue is richer in mucin than old. HALLIBURTON[2] found an average of 7.66 p. m. mucin in the skin of very young children and only 3.85 p. m. in the skin of adults. In so-called myxœdema, in which a reformation of the connective tissue of the skin takes place, the quantity of mucin is also increased.

II. Cartilage.

Cartilaginous tissue consists of cells and an originally hyaline matrix, which, however, may become changed in such wise that there appears in it a network of elastic fibres or connective-tissue fibrils.

Those cells that offer great resistance to the action of alkalies and acids have not been carefully studied. According to former views, the matrix was considered as consisting of a body analogous to collagen, so-called *chondrigen*, which under similar conditions passes, like collagen, into a corresponding gelatin called *chondrin* or cartilage-gelatin. The recent investigations of MOROCHOWETZ[3] and others, but especially those of C. TH. MÖRNER,[4] have shown that the matrix of the cartilage consists of a mixture of collagen with other bodies.

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[1] Zeitschr. f. physiol. Chem., Bd. 18, S. 250.

[2] Mucin in Myxœdema. Further Analyses. Kings College. Collected Papers No. 1, 1893.

[3] Verhandl. d. naturhist.-med. Vereins zu Heidelberg, Bd. 1, Heft 5.

[4] Skand. Arch. f. Physiol., Bd. 1.

The tracheal, thyroideal, cricoidal, and arytenoidal cartilages of full-grown cattle contain, according to MÖRNER, four constituents in the matrix, namely, *chondromucoid*, *chondroitin-sulphuric acid*, *collagen*, and an *albuminoid*.

Chondromucoid. This body, according to MÖRNER, has the composition C 47.30, H 6.42, N 12.58, S 2.42, O 31.28%. Sulphur is in part loosely combined and may be split off by the action of alkalies, and a part separates as sulphuric acid when boiled with hydrochloric acid. Chondromucoid is decomposed by dilute alkalies and yields alkali albuminate, peptone substances, chondroitin-sulphuric acid, alkali sulphides, and some alkali sulphates. On boiling with acids it yields acid albuminate, peptone substances, chondroitin-sulphuric acid, and on account of the further decomposition of this last body sulphuric acid and a reducing substance are formed. According to SCHMIEDEBERG[1] chondromucoid is a combination of chondroitin-sulphuric acid with proteid.

Chondromucoid is a white, amorphous, acid-reacting powder which is insoluble in water, but dissolves easily on the addition of a little alkali. This solution is precipitated by acetic acid in great excess and by small quantities of mineral acids. The precipitation may be retarded by neutral salts or by chondroitin-sulphuric acid. The solution containing NaCl and acidified with HCl is not precipitated by potassium ferrocyanide. Precipitants for chondromucoid are alum, ferric chloride, sugar of lead or basic lead acetate. Chondromucoid is not precipitated by tannic acid, and it may by its presence prevent the precipitation of gelatin by this acid. It gives the usual color reactions for proteids, namely, with nitric acid, with copper sulphate and alkali, with MILLON'S and ADAMKIEWICZ'S reagents.

Chondroitin-sulphuric Acid, CHONDROITIC ACID. This acid, which was first prepared pure from cartilage by C. TH. MÖRNER and identified by him as an ethereal sulphuric acid, occurs according to MÖRNER[2] in all varieties of cartilage and also in the tunica intima of the aorta. ODDI[3] has also found it in livers with amyloid degeneration. According to SCHMIEDEBERG the acid has the formula $C_{18}H_{27}NSO_{17}$. In regard to the chemical constitution of this acid the investigations of SCHMIEDEBERG have led to the following:

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[1] Arch. f. exp. Path. u. Pharm., Bd. 28.

[2] Upsala Läkarefs Förh., Bd. 29.

[3] Arch. f. exp. Path. u. Pharm., Bd. 33.

As first products this acid yields sulphuric acid and a nitrogenous substance, *chondroitin*, according to the following equation:

$$C_{18}H_{27}NSO_{17} + H_2O = H_2SO_4 + C_{18}H_{27}NO_{14}.$$

Chondroitin, which is similar to gum arabic and which is a monobasic acid, yields acetic acid and a new nitrogenous substance, *chondrosin*, as cleavage products, on decomposition with dilute mineral acids:

$$C_{18}H_{27}NO_{14} + 3H_2O = 3C_2H_4O_2 + C_{12}H_{21}NO_{11}$$

Chondrosin, which is also a gummy substance soluble in water, is a monobasic acid and reduces copper oxide in alkaline solution even more strongly than dextrose. It is dextrogyrate and represents the reducing substance obtained by previous investigators in an impure form on boiling cartilage with an acid. The products obtained on decomposing chondrosin with barium hydrate tend to show that chondrosin contains the atomic groups of glycuronic acid and glucosamine.

Chondroitin-sulphuric acid appears as a white amorphous powder, which dissolves very easily in water, forming an acid solution and, when sufficiently concentrated, a sticky liquid similar to a solution of gum arabic. Nearly all of its salts are soluble in water. The neutralized solution is precipitated by tin chloride, basic lead acetate, neutral ferric chloride, and by alcohol in the presence of a little neutral salt. The solution, on the other hand, is not precipitated by acetic acid, tannic acid, potassium ferrocyanide and acid, sugar of lead, mercuric chloride, or silver nitrate. Acidified solutions of chondroitin-sulphates cause a precipitation when added to solutions of gelatin or proteid.

Chondromucoid and chondroitin-sulphuric acid may be prepared according to MÖRNER[1] by exacting finely cut cartilage with water, which dissolves the preformed chondroitin-sulphuric acid besides some chondromucoid. In this watery extract the chondroitin-sulphuric acid prevents the precipitation of the chrodromucoid by means of an acid. If 2–4 p. m. HCl is added to this watery extract and warmed on the water-bath, the chondromucoid gradually separates, while the chondroitin-sulphuric acid and the rest of the chondromucoid remain in the filtrate. If the cartilage, which has been lixiviated, at the temperature of the body, with water, is extracted with hydrochloric acid of 2–3 p. m. until the collagen is converted into gelatin and dissolved, the remaining chondromucoid may be removed from the insoluble residue by dilute alkali and precipitated from the alkaline extract by an acid. It may be purified by repeated solution in water with the aid of a little alkali, precipitating by an acid and then treating with alcohol and ether.

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[1] L. c.

The pre-existing chondroitin-sulphuric acid, or that formed by the decomposition of chondromucoid, is obtained by lixiviating the cartilage with a 5% caustic-alkali solution. The alkali albuminate formed by the decomposition of the chondromucoid can be removed from the solution by neutralization, then the peptone precipitated by tannic acid, the excess of this acid removed with sugar of lead, and the lead separated from the filtrate by H_2S. If further purification is necessary, the acid is precipitated with alcohol, the precipitate dissolved in water, this solution dialyzed and precipitated again with alcohol,—this solution in water and precipitating with alcohol being repeated a few times,—and lastly the acid is treated with alcohol and ether.

SCHMIEDEBERG[1] prepared the acid from the septum narium of the pig according to the following method: The finely divided cartilage is first exposed to artificial pepsin digestion and then carefully washed with water and the insoluble residue treated with 2–3% hydrochloric acid. This cloudy liquid containing hydrochloric acid is precipitated with alcohol (about ½ vol.) and the clear filtrate treated with absolute alcohol and some ether. The precipitate, consisting chiefly of a combination or a mixture of chondroitin-sulphuric acid and gelatin peptone (pepto-chondrin), is first washed with alcohol and then with water. It is then dissolved in alkaline water and the basic alkali combination precipitated from this solution by the addition of alcohol, whereby the gelatine-peptone alkali remains in solution. The precipitate is purified by repeated solution in alkaline water and precipitated by alcohol. To obtain chondroitin-sulphuric acid entirely free from chondroitin it is more advantageous to prepare the potassium-copper combination of the acid from the alkaline solution by the alternate addition of copper acetate and caustic potash and precipitating with alcohol. The reader is referred to the original article for more details.

The *collagen* of the cartilage gives, according to MÖRNER, a gelatin which contains only 16.4% N and which can hardly be considered identical with ordinary gelatin.

In the above-mentioned cartilages of full-grown animals the chondroitin-sulphuric acid and chondromucoid, perhaps also the collagen, are found surrounding the cells as round balls or lumps. These balls (MÖRNER'S *chondrin-balls*), which give a blue color with methyl-violet, lie in the meshes of a trabecular structure, which is colored when brought in contact with tropæolin.

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[1] Arch. f. Exp. Path. u. Pharm., Bd. 28.

The *albuminoid* is a nitrogenized body which contains loosely combined sulphur. It is soluble with difficulty in acids and alkalies, and resembles keratin in many respects, but differs from it by being soluble in gastric juice. In other respects it is more similar to elastin, but differs from this substance by containing sulphur. This albuminoid gives the color reactions of the albuminous bodies.

The preparation of cartilage-gelatin and albuminoid may be performed according to the following method of MÖRNER: First remove the chondromucoid and chondroitin-sulphuric acid by extraction with dilute caustic potash (0.2–0.5%), remove the alkali from the remaining cartilage by water, and then boil with water in a PAPIN's digestor. The collagen passes into solution as gelatin, while the albuminoid remains undissolved (contaminated by the cartilage-cells). The gelatin may be purified by precipitating with sodium sulphate, which must be added to saturation in the faintly acidified solution, redissolving the precipitate in water, dialyzing well, and precipitating with alcohol.

According to MÖRNER, no albuminoid is found in young cartilage, but only the three first-mentioned constituents. Nevertheless the young cartilage contains about the same amounts of nitrogen and mineral substances as the old.

HOPPE-SEYLER[1] found in fresh human rib-cartilage 676.7 p. m. water, 301.3 p. m. organic and 22 p. m. inorganic substance, and in the cartilage of the knee-joint 735.9 p. m. water, 248.7 p. m. organic and 15.4 p. m. inorganic substance. PICKARDT[2] found 402–574 p. m. water and 72.86 p. m. ash (no iron) in the laryngeal cartilage of oxen. The ash of cartilage contains considerable amounts (even 800 p. m.) of alkali sulphate, which probably does not exist originally as such, but is produced in great part by the calcination of the chondroitin-sulphuric acid and the chondromucoid. The analyses of the ash of cartilage therefore cannot give a correct idea of the quantity of mineral bodies existing in this substance. PETERSEN and SOXHLET[3] have found 940 p. m. NaCl in the ash from the cartilage of a shark, and such cartilage can scarcely contain quantities of chondromucoid or chondroitin-sulphuric acid worth mentioning. The cartilage of the ray (*Raja batis* LIN.), which has been investigated by LÖNNBERG,[1] contains no albuminoid and only a little chondromucoid, but a large proportion of chondroitin-sulphuric acid and collagen.

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[1] Cited from Kühne, Lehrb. d. physiol. Chem., 1868, S. 387.

[2] Centralbl. f. Physiol., Bd. 6, S. 735.

[3] Journ. f. prakt. Chem. (N. F.), Bd. 7.

The Cornea. The corneal tissue, which is considered by many investigators to be related to cartilage in a chemical sense, contains traces of proteid and a *collagen* as chief constituent, which C. TH. MÖRNER[2] claims contains 16.95% N. According to him it also contains a *mucoid* which has the composition C 50.16, H 6.97, N 12.79, and S 2.07%. On boiling with dilute mineral acid this mucoid yields a reducing substance. The globulins found by other investigators in the cornea are not derived from the matrix, according to MÖRNER, but from the layer of epithelium. According to MÖRNER, DESCEMET'S membrane consists of *membranin* (page 47), which contains 14.77% N and 0.90% S.

In the cornea of oxen HIS[3] found 758.3 p. m. water, 203.8 p. m. gelatin-forming substance, 28.4 p. m. other organic substance, besides 8.1 p. m. soluble and 1.1 p. m. insoluble salts.

III. Bone.

The bony structure proper, when free from other formations occurring in bones, such as marrow, nerves, and blood-vessels, consists of cells and a matrix.

The *cells* have not been closely studied in regard to their chemical constitution. On boiling with water they yield no gelatin. They contain no keratin, which is not usually present in the bony structure (HERBERT SMITH[4]), but they may contain a substance which is similar to elastin.

The *matrix* of the bony structure contains two chief constituents, namely, an organic substance, *ossein*, and the so-called *bone-earths*, lime-salts, enclosed in or combined with it. If bones are treated with dilute hydrochloric acid at the ordinary temperature, the lime-salts are dissolved and the ossein remains as an elastic mass, preserving the shape of the bone. This ossein is generally considered identical with the collagen of the connective tissue. The ossein in the bones of certain aquatic fowls and fishes can hardly be considered identical with this collagen (FREMY[5]).

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[1] Upsala Läkaref. Förh., Bd. 24; also Maly's Jahresber., Bd. 19, S. 325.

[2] Zeitschr. f. physiol. Chem., Bd. 18.

[3] Cited from Gamgee, Physiol. Chem., 1880, p. 451.

[4] Zeitschr. f. Biologie, Bd. 19.

[5] Annal. de Chim. et de phys. (3), Tome 43, and Compt. rend., Tome 39.

The inorganic constituents of the bony structure, the so-called *bone-earths*, which remain after the complete calcination of the organic substance as a white, brittle mass, consist chiefly of calcium and phosphoric acid, but also contain carbon dioxide and, in smaller amounts, magnesium, chlorine, and fluorine. Alkali sulphate and iron, which have been found in bone-ash, do not seem to belong exactly to the bony substance, but to the nutritive fluids or to the other constituents of bones. According to GABRIEL[1] potassium and sodium are essential constituents of bone-earth.

The opinions of investigators differ somewhat as to the manner in which the mineral bodies of the bony structure are combined with each other. Chlorine and fluorine are present in the same form as in apatite ($CaFl_2,3Ca_3P_2O_8$). If we eliminate the magnesium, the chlorine, and the fluorine, the last, according to GABRIEL, occurring only as traces, the remaining mineral bodies form the combination $3(Ca_3P_2O_8)CaCO_3$. According to GABRIEL the simplest expression for the composition of the ash of bones and teeth is ($Ca_3(PO_4)_2 + Ca_5HP_3O_{13} + Aq$), in which 2–3% of the lime is replaced by magnesia, potash, and soda, and 4–6% of the phosphoric acid by carbon dioxide, chlorine, and fluorine.

Analyses of bone-earths have shown that the mineral constituents exist in rather constant proportions, which is nearly the same in different animals. As example of the composition of bone earth we give here the analyses of ZALESKY.[2] The figures represent parts per thousand.

	Man.	Ox.	Tortoise.	Guinea-pig.
Calcium phosphate, $Ca_3P_2O_8$	838.9	860.9	859.8	873.8
Magnesium phosphate, $Mg_3P_2O_8$	10.4	10.2	13 6	10.5
Calcium combined with CO_2, Fl, and Cl.	76.5	73.6	63.2	70.3
CO_2	57.3	62.0	52.7	....
Chlorine	1.8	2.0	....	1.3
Fluorine	2.3	3.0	2.0	....

Some of the CO_2 is always lost on calcining, so that the bone-ash does not contain the entire CO_2 of the bony substance.

AD. CARNOT[3] found the following composition for the bone-ash of man, ox, and elephant:

	Man.		Ox.	Elephant.
	Femur (body).	Femur (head).	Femur.	Femur.
Calcium phosphate	874.5	878.7	857.2	900.3
Magnesium phosphate	15.7	17.5	15.3	19.6
Calcium fluoride	3.5	3.7	4.5	4.7
Calcium chloride	2.3	3.0	3.0	2.0
Calcium carbonate	101.8	92.3	119.6	72.7
Iron oxide	1.0	1.3	1.3	1.5

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[1] Zeitschr. f. physiol. Chem., Bd. 18.

[2] Hoppe-Seyler, Med. chem. Untersuch., S. 19.

[3] Comp. rend., Tome 114.

The quantity of organic substance in the bones, calculated from the loss of weight in burning, varies somewhat between 300 and 520 p. m. This variation may in part be explained by the difficulty in obtaining the bony substance entirely free from water, and partly by the very variable amount of blood-vessels, nerves, marrow, and the like in different bones. The unequal amounts of organic substance found in the compact and in the spongy parts of the same bone, as well as in bones at different periods of development in the same animal, depend probably upon the varying quantities of these above-mentioned formations. *Dentin*, which is comparatively pure bony structure, contains only 260–280 p. m. organic substance, and HOPPE-SEYLER[1] therefore thinks it probable that entirely pure bony substance has a constant composition and contains only about 250 p. m. organic substance. The question whether these substances are chemically combined with the bone-earths or only intimately mixed has not been decided.

The nutritive fluids which circulate through the bones have not been isolated, and we only know that they contain some proteid and some NaCl and alkali sulphate. The yellow marrow contains chiefly fat, which consists of olein, palmitin, and stearin. Proteid has been found especially in the so-called red marrow of the spongy bones. According to FORREST[2] the proteid consists of a globulin coagulating at 47–50° C., and a nucleo-albumin, besides traces of albumin. Besides this the marrow contains so-called extractive bodies, such as lactic acid, hypoxanthin, and cholesterin, but mostly bodies of an unknown character.

The diverse quantitative composition of the various bones of the skeleton depends probably on the varying quantities of other formations, such as marrow, blood-vessels, etc., they contain. The same reason explains, to all appearances, the larger quantity of organic substance in the spongy parts of the bones as compared with the more compact parts. SCHRODT[3] has made comparative analyses of different parts of the skeleton of the same animal (dog), and has found an essential difference. The quantity of water in the fresh bones varies between 138 and 443 p. m. The bones of the extremities and the skull contain 138–222, the vertebræ 168–443, and the ribs 324–356 p. m. water. The quantity of fat varies between 13 and 269 p. m. The largest amount of fat, 256–269 p. m., is found in the long tubular bones, while only 13–175 p. m.

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[1] Physiol. Chem., S. 102–104.

[2] Journal of Physiol., Vol. 17.

[3] Landwirthsch. Versuchsstat., Bd. 19. Cited from Maly's Jahresber., Bd. 6.

fat is found in the small short bones. The quantity of organic substance, calculated from fresh bones, was 150–300 p. m., and the quantity of mineral substances 290–563 p. m. Contrary to the general supposition the greatest amount of bone-earths was not found in the femur, but in the first three cervical vertebræ. In geese the largest amount of bone-earth was found in the humerus (HILLER[1]).

We do not possess trustworthy statements in regard to the composition of bones at different ages. According to the analyses of E. VOIT[2] of bones of dogs and of BRUBACHER[3] of bones of children, we learn that the skeleton becomes poorer in water and richer in ash with increase in age. GRAFFENBERGER[4] has found in rabbits 6½–7½ years old that the bones contained only 140–170 p. m. water, while the bones of the full-grown rabbit 2–4 years old contained 200–240 p. m. The bones of old rabbits contain more carbon dioxide and less calcium phosphate.

The composition of bones of animals of different species is but little known. The bones of birds contain, as a rule, somewhat more water than those of mammalia, and the bones of fishes contain the largest quantity of water. The bones of fishes and amphibians contain a greater amount of organic substance. The bones of pachyderms and cetaceans contain a large proportion of calcium carbonate; those of granivorous birds always contain silicic acid. The bone-ash of amphibians and fishes contains sodium sulphate. The bones of fishes seem to contain more soluble salts than the bones of other animals.

A great many experiments have been made to determine the exchange of material in the bones—for instance, with food rich in lime and with food deficient in lime—but the results have always been doubtful or contradictory. The attempts, also, to substitute other alkaline earths or clay for the lime of the bones have given contradictory results. WEISKE[5] has shown by experiments on not quite full grown and on young and still rapidly growing rabbits that on feeding with oats, which are poor in acid and lime, and simultaneously magnesium or strontium carbonate, that these in part pass into the skeleton; but a physiological replacement of lime by magnesium or strontium is not to be expected. On the administration of madder the bones of the animal are found to be colored

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[1] Landwirthsch. Versuchsstat., Bd. 31. Cited from Maly's Jahresber., Bd. 14.

[2] Zeitschr. f. Biologie, Bd. 16.

[3] *Ibid.*, Bd. 27.

[4] Landwirthsch. Versuchsstat., Bd. 39. Cited from Maly's Jahresber., Bd. 21.

[5] Zeitschr. f. Biologie, Bd. 31.

red after a few days or weeks; but these experiments have not led to any positive conclusion in regard to the growth or metabolism in the bones.

Under pathological conditions, as in rachitis and softening of the bones, an ossein has been found which does not give any typical gelatin on boiling with water. Otherwise pathological conditions seem to affect chiefly the quantitative composition of the bones, and especially the relationship between the organic and the inorganic substance. In exostosis and osteosclerosis the quantity of organic substance is generally increased. Attempts have been made to produce rachitis in animals by the use of food deficient in lime. From experiments on fully developed animals contradictory results have been obtained. In young, undeveloped animals ERWIN VOIT[1] produced, by lack of lime-salts, a change similar to rachitis. In full-grown animals the bones were changed after a long time because of the lack of lime-salts in the food, but did not become soft, only thinner (osteoporosis). The experiments of removing the lime-salts from the bones by the addition of lactic acid to the food have led to no positive results (HEITZMANN,[2] HEISS,[3] BAGINSKY[4]). WEISKE,[5] on the contrary, has shown, by administering dilute sulphuric acid or monosodium phosphate with the food (presupposing that the food gave no alkaline ash) to sheep and rabbits, that the quantity of mineral bodies in the bones might be diminished. A few investigators are of the opinion that in rachitis, as in osteomalacosis, a solution of the lime-salts by means of lactic acid takes place. This was suggested by the fact that O. WEBER and C. SCHMIDT[6] found lactic acid in the cyst-like, altered bony substance in osteomalacia.

Well-known investigators have disputed the possibility of the lime-salts being washed from the bones in osteomalacosis by means of lactic acid. They have given special prominence to the fact that the lime-salts held in solution by the lactic acid must be deposited on neutralization of the acid by the alkaline blood. This objection is not very important, as the alkaline stream of blood has the property to a high degree of holding earthy phosphates in solution, which can be easily proved. The recent investigations of LEVY[1] contradict the statement as to the solution of the lime-salts by lactic acid in osteomalacia. He has found that the normal relationship $6PO_4 : 10Ca$ is retained in all parts of the bones in osteomalacia, which would not be the case if the bone-earths were dissolved by an acid. The decrease in phosphate occurs in the same quantitative relationship as the carbonate, and according to LEVY in osteomalacia the exhaustion of the bone takes place by a decalcification in which one molecule of phosphate carbonate after the other is removed.

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[1] L. c.

[2] Maly's Jahresber., Bd. 3, S. 229.

[3] Zeitschr. f. Biologie, Bd. 12.

[4] Virchow's Arch., Bd. 87.

[5] Landwirthsch. Versuchsstat., Bdd. 39 and 40. Cited from Maly's Jahresber., Bd. 22.

-Besanez, Lehrb. d. physiol. Chem., 4. Aufl., S. 636.

In rachitis the quantity of organic matter has been found to vary between 664 and 811 p. m. The quantity of inorganic substance was 189–336 p. m. These figures refer to the dried substance. According to BRUBACHER[2] rachitic bones are richer in water than the bones of healthy children, and poorer in mineral bodies, especially calcium phosphate. In opposition to rachitis, osteomalacosis is often characterized by the considerable amount of fat in the bones, 230–290 p. m.; but as a rule the composition varies so much that the analyses are of little value.

The **tooth-structure** is nearly related, from a chemical standpoint, to the bony structure.

Of the three chief constituents of the teeth, dentin, enamel, and cement, the last-mentioned, the *cement*, is to be considered as true bony structure, and as such has already been spoken of to a certain extent. *Dentin* has the same composition as the bony structure, but contains somewhat less water. The organic substance yields gelatin on boiling; but the dental tubes are not dissolved, therefore they cannot consist of collagen. In dentin 260–280 p. m. organic substance has been found. *Enamel* is an epithelium formation containing a large proportion of lime-salts. The organic substance of the enamel does not yield any gelatin. Completely developed enamel contains the least water, the greatest quantity of mineral substances, and is the hardest of all the tissues of the body. In full-grown animals it contains hardly any water, and the quantity of organic substance amounts to only 20–40 p. m. The relative amounts of calcium and phosphoric acid are, according to the analyses of HOPPE-SEYLER, about the same as in bone-earths. The quantity of chlorine is according to HOPPE-SEYLER[3] remarkably high, 0.3–0.5%.

[1] Zeitschr. f. physiol. Chem., Bd. 19.

[2] Zeitschr. f. Biologie, Bd. 27.

[3] L. c.

CARNOT,[1] who has investigated the dentin from elephants, has found 4.3 p. m. calcium fluoride in the ash. In ivory he found only 3.0 p. m. Dentin from elephants is rich in magnesium phosphate, which is more marked in ivory.

According to GABRIEL the amount of fluorine is very small and amounts to 1 p. m. in ox-teeth. It is greater in the teeth and enamel than in the bones. According to GABRIEL a strikingly small portion of the lime of the enamel is replaced by magnesia, while in the teeth it is considerable.

IV. The Fatty Tissue.

The membranes of the fat-cells withstand the action of alcohol and ether. They are not dissolved by acetic acid nor by dilute mineral acids, but are dissolved by artificial gastric juice. They may possibly consist of a substance closely related to elastin. The contents of the fat-cells are fluid during life, but solidify after death and become more or less solid, depending upon the character of the fats. Besides fat, the fat-cells contain a yellow pigment which in emaciation does not disappear so rapidly as the fat; and this is the reason that the subcutaneous cellular tissue of an emaciated corpse has a dark orange-red color. The cells deficient in or nearly free from fat, which remain after the complete disappearance of the latter, seem to have an albuminous protoplasm rich in water.

The less water the fatty tissue contains the richer it is in fat. SCHULTZE and REINECKE[2] found in 1000 parts:

	Water.	Membrane.	Fat.
Fatty tissue of oxen	99.7	16.6	883.7
" " " sheep...........	104.8	16.4	878.8
" " " pigs.....	64.4	13.6	922.0

The fat contained in the fat-cells consists chiefly of triglycerides of stearic, palmitic, and oleic acids. Besides these, especially in the less solid kinds of fats, there are glycerides of caproic, valerianic, and other fatty acids which have not been so closely investigated. In all animal fats there are besides these, as HOFMANN[3] has shown, also free, non-volatile fatty acids, although in very small amounts.

The more solid varieties of fat of the adipose tissue consist, as previously stated (Chapter IV), in great part of stearin and palmitin, while the less solid fats have a greater quantity of olein. Human fat is as a rule rich in olein. The fatty tissue of cold-blooded animals is especially rich in olein.

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[1] L. c.

[2] Ann. d. Chem. u. Pharm., Bd. 142.

[3] Ludwig-Festschrift, 1874.

The properties of fats in general and the three most important varieties of fat have already been treated of in a previous chapter, hence the formation of the adipose tissue is of chief interest at this time.

The formation of fat in the organism may occur in various ways. The fat of the animal body may consist partly of absorbed fat of the food deposited in the tissues and partly of fat formed in the organism from other bodies, such as proteids or carbohydrates.

That the fat of the food which is absorbed in the intestinal canal may be retained by the tissues has been shown in several ways. RADZIEJEWSKY,[1] LEBEDEFF,[2] and MUNK[3] have fed dogs with various fats, such as linseed-oil, mutton-tallow, and rape-seed-oil, and have afterwards found the administered fat in the tissues. HOFMANN[4] starved dogs until they appeared to have lost their fat, and then fed them upon large quantities of fat and only little proteids. When the animals were killed he found so large a quantity of fat that it could not have been formed from the administered proteids alone, but the greatest part must have been derived from the fat of the food. PETTENKOFER and VOIT[5] arrived at similar results in regard to the behavior of the absorbed fats in the organism, though their experiments were of another kind. MUNK[6] has found that on feeding with free fatty acids these are deposited in the tissues, not, however, as such; but they are transformed by synthesis with glycerin into neutral fats on their passage from the intestine to the thoracic duct. According to EWALD,[7] such a synthesis may be produced by the mucous membrane of the intestine.

Proteids and carbohydrates are considered as the mother-substance of the fats formed in the organism.

The formation of the so-called CORPSE-WAX, ADIPOCERE, which consists of a mixture of fatty acids, ammonia, and lime-soaps, from parts of the corpse rich in proteids, is sometimes given as a proof of the *formation of fats from proteids*. The provableness of this observation has, however, been disputed, and many other explanations of the formation of this substance have been offered. According to the recent experiments of KRATTER[1] and K. B. LEHMANN[2] it seems as if it were possible by experimental means to convert animal tissue rich in proteids (muscles) into adipocere by the continuous action of water. Irrespective of this, SALKOWSKI[3] has shown recently that in the formation of adipocere the fat itself takes part in that the olein decomposes with the formation of solid fatty acids; still it must be considered that lower organisms undoubtedly take part in its formation. The formation of adipocere as a proof of the formation of fat from proteids is disputed by many investigators for this and other reasons.

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[1] Virchow's Arch., Bd. 43.

[2] Pflüger's Arch., Bd. 31.

[3] Virchow's Arch., Bd. 95.

[4] Zeitschr. f. Biologie, Bd. 8.

[5] *Ibid.*, Bd. 9.

[6] Virchow's Arch., Bd. 80.

[7] Du Bois-Reymond's Arch., 1883.

Fatty degeneration is another proof of the formation of fat from proteids. From the investigations of BAUER[4] on dogs and LEO[5] on frogs we must admit that at least in acute poisoning by phosphorus a fatty degeneration with the formation of fat from proteids takes place. Still investigators are not unanimous on this point, and PFLÜGER[6] has especially raised important objections to these experiments.

As a more direct proof of fat-formation from proteids the investigations of PETTENKOFER and VOIT[7] are often quoted. These investigators fed dogs with large quantities of meat containing the least possible proportion of fat, and found all of the nitrogen in the excreta, but only a part of the carbon. As an explanation of these conditions it has been assumed that the proteid of the organism splits into a nitrogenized and a non-nitrogenized part, the former changing into the nitrogenized final product, urea, the other, on the contrary, being retained in the organism as fat (PETTENKOFER and VOIT).

PFLÜGER[8] has arrived at the following conclusion by an exhaustive criticism of PETTENKOFER and VOIT'S experiments and a careful recalculation of their balance-sheet, namely, that these very meritorious investigations, which were continued for a series of years, were subject to such great defects that they are not conclusive as to the formation of fat from proteids. He especially emphasizes the fact that these investigators started from a wrong assumption as to the elementary composition of the meat, and that the quantity of nitrogen assumed by them was too low and the quantity of carbon too high. The relationship of nitrogen to carbon in meat poor in fat was assumed by VOIT to be as 1 : 3.68, while according to PFLÜGER it is 1 : 3.22 for fat-free meat after deducting the glycogen and 1 : 3.28 according to RUBNER without deducting the glycogen. On recalculating the experiments using these coefficients, PFLÜGER has arrived at the conclusion that the assumption as to the formation of fat from proteids finds no support in these experiments.

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[1] Zeitschr. f. Biologie, Bd. 16.

[2] Sitzungsber. d. Würzb. phys.-med. Gesellsch., 1888.

[3] Zur Kenntniss der Fettwachsbildung. Virchow's Festschrift, 1891.

[4] Zeitschr. f. Biologie, Bd. 7.

[5] Zeitschr. f. physiol. Chem., Bd. 9.

[6] Pflüger's Arch., Bd. 51. This contains also the most important literature on the formation of fat from proteids.

[7] Liebig's Annal., Suppl. Bd. 2, and Zeitschr. f. Biologie, Bdd. 5, 6, and 7.

[8] L. c.

ERWIN VOIT[1] on recalculating these older experiments finds, contrary to the above objections, that at least in a few cases a deposit of carbon originating from the proteids takes place in the body. He has also made new experiments, which demonstrate, according to him, that the administration of an excess of meat causes a deposition of a part of the carbon as a non-nitrogenous combination (probably fat) in the body.

Another more direct proof for the formation of fat from proteids has been given by HOFMANN.[2] He experimented with fly-maggots. A number of these were killed and the quantity of fat determined. The remainder were allowed to develop in blood whose proportion of fat had been previously determined, and after a certain time they were killed and analyzed. He found in them from 7 to 11 times as much fat as in the maggots first analyzed and the blood together contained. PFLÜGER[3] has made the objection that a considerable number of lower fungi develop in the blood under these conditions, and these serve as food for the maggots and in whose cell-body they form fats and carbohydrates from the different constituents of the blood and their decomposition products.

The views are therefore very diverse in regard to the conclusiveness of these experiments as to the formation of fat from proteids. The possibility of a formation of fat from proteids can hardly be disputed by any investigator.

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[1] Münch. med. Wochenschr., 1892, No. 26. Cited from Maly's Jahresber., Bd. 22.

[2] Zeitschr. f. Biologie, Bd. 8.

[3] L. c.

If we admit of the possibility of the formation of fat from proteids, still we must also admit that we do not know anything with positiveness in regard to the chemical processes which take place. DRECHSEL,[1] mindful of the products which are formed by the decomposition of proteids with barium hydrate, has called attention to the fact that the proteid molecule probably originally contains no radical with more than six or nine carbon atoms. If fat is formed from proteid in the animal body, then, according to DRECHSEL, such formation is not a splitting off of fat from the proteids, but rather a synthesis from primarily formed splitting products of proteids which are deficient in carbon.

The *formation of fat from carbohydrates* in the animal body was first suggested by LIEBIG. This was combated for some time, and until lately it was the general opinion that a direct formation of fat from carbohydrates had not been proven, but also that it was improbable. The undoubtedly great influence of the carbohydrates on the formation of fat as observed and proven by LIEBIG was explained by the statement that the carbohydrates were consumed instead of the absorbed fat or that derived from the proteids, hence they have a sparing action on the fat. By means of a series of nutrition experiments with foods especially rich in carbohydrates, LAWES and GILBERT,[2] SOXHLET,[3] TSCHERWINSKY,[4] MEISSL and STROMER[5] (on pigs), B. SCHULTZE,[6] CHANIEWSKI,[7] E. VOIT and C. LEHMANN[8] (on geese), I. MUNK[9] and M. RUBNER[10] (on dogs) apparently prove that a direct formation of fat from carbohydrates does actually occur. The processes by which this formation takes place are still unknown. As the carbohydrates do not contain as complicated carbon chains as the fats, the formation of fat from carbohydrates must consist of a synthesis, in which the group CHOH is converted into CH_2; also a reduction must take place.

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[1] Ladenburg's Handwörterbuch der Chem., Bd. 3, S. 543.

[2] Phil. Transactions, 1859, part 2.

[3] See Maly's Jahresber., Bd. 11, S. 51.

[4] Landwirthsch. Versuchsstat., Bd. 29. Cited from Maly's Jahresber., Bd. 13.

[5] Wien. Sitzungsber., Bd. 88. Abth. 3.

[6] Maly's Jahresber., Bd. 11. S. 47.

[7] Zeitschr. f. Biologie, Bd. 20.

[8] See C. v. Voit, Sitzungsber. d. k. bayer. Akad. d. Wissensch. 1885.

[9] Virchow's Arch., Bd. 101.

[10] Zeitschr. f. Biologie, Bd. 22.

When food contains an excess of fat the superfluous amount is stored up in the fatty tissue, and on partaking of food deficient in fat this accumulation is quickly exhausted. There is perhaps not one of the various tissues that decreases so much in starvation as the fatty tissue. The organism, then, possesses in this tissue a depot where there is stored during proper alimentation a nutritive substance of great importance in the development of heat and vital force, which substance, on insufficient nutrition, is given off as may be needed. On account of their low conducting power the fatty tissues become of great importance in regulating the loss of heat from the body. They also serve to fill cavities and as a protection and support to certain internal organs.

CHAPTER XI.

MUSCLE.

Striated Muscles.

IN the study of the muscles the chief problem for physiological chemistry is to isolate their different morphological elements and to investigate each element separately. By reason of the complicated structure of the muscles this has been thus far almost impossible, and we must be satisfied at the present time with a few microchemical reactions in the investigation of the chemical composition of the muscular fibres.

Each muscle-tube or muscle-fibre consists of a sheath, the SARCOLEMMA, which seems to consist of a substance similar to elastin, and a contents containing a large proportion of PROTEIDS. This last, which in life possesses the power of contractility, has in the inactive muscle an alkaline reaction, or, more correctly speaking, an amphoteric reaction with a predominating action on red litmus-paper. RÖHMANN[1] has found that the fresh, inactive muscle shows an alkaline reaction with red lacmoid and an acid reaction with brown turmeric. From the behavior of these coloring matters with various acids and salts he concludes that the alkalinity of the fresh muscle with lacmoid is due to sodium bicarbonate, diphosphate, and probably also to an alkaline combination of proteid bodies, and the acid reaction with turmeric, on the contrary, to monophosphate chiefly. The dead muscle has an acid reaction, or more correctly the acidity with turmeric increases on the decease of the muscle and the alkalinity with lacmoid decreases. The difference depends on the presence of a larger quantity of monophosphate in the dead muscle, and according to RÖHMANN free lactic acid is found in neither the one case nor the other.

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[1] The various statements in regard to the reaction of the muscles and the cause thereof are disputed. See Röhmann, Pflüger's Arch., Bdd. 50 and 55; Heffter, Arch. f. exp. Path. u. Pharm., Bd. 31.

If we disregard the somewhat disputed statements relative to the finer structure of the muscles, we can differentiate in the striated muscles between the two chief components, the doubly refracting—*anisotropous*—and the singly refracting—*isotropous*—substance. If the muscular fibres are treated with reagents which dissolve proteids, such as dilute hydrochloric acid, soda solution, or gastric juice, they swell greatly and break up into "BOWMAN'S *disks*." By the action of alcohol, chromic acid, boiling water, or in general such reagents as cause a shrinking, the fibres split longitudinally into fibrils; and this behavior shows that several chemically different substances of various solubilities enter into the construction of the muscular fibres.

The proteid myosin is generally considered as the chief constituent of the diagonal disks, while the isotropous substance contains the chief mass of the other proteids of the muscles as well as the chief portion of the extractives. According to the observations of DANILEWSKY,[1] and recently confirmed by J. HOLMGREN,[2] myosin may be completely extracted from the muscle without changing its structure, by means of a 5% solution of ammonium chloride. DANILEWSKY claims that another proteid-like substance, insoluble in ammonium chloride and only swelling up therein, enters essentially into the structure of the muscles. The proteids, which form the chief part of the solids of the muscles, are of the greatest importance.

Proteids of the Muscles.

Like the blood which contains a fluid, the blood-plasma, which spontaneously coagulates, separating fibrin and yielding blood-serum, so also the living muscle contains, as first shown by KÜHNE, a spontaneously coagulating liquid, the muscle-plasma, which coagulates quickly, separating a proteid body, myosin, and yielding also a serum. That liquid which is obtained by pressing the living muscle is called *muscle-plasma*, while that obtained from the dead muscle is called *muscle-serum*. These two fluids contain different albuminous bodies.

Muscle-plasma was first prepared by KÜHNE[3] from frog-muscles, and later by HALLIBURTON[1] according to the same method from the muscles of warm-blooded animals, especially rabbits. The principle of this method is as follows: The blood is removed from the muscles immediately after the death of the animal by passing through them a strongly cooled common-salt solution of 5–6 p. m. Then the quickly cut muscles are immediately thoroughly frozen so that they can be ground in this state to a fine mass—"muscle-snow." This pulp is strongly pressed in the cold, and the liquid which exudes, the muscle-plasma, is faintly yellowish in color, alkaline, and spontaneously but slowly coagulates at a little above 0° C., but very quickly at the temperature of the body. In the muscle-plasma of the frog the reaction does not change immediately with the coagulation, but the alkaline reaction is gradually changed into an acid one. The liquid which exudes from the clot, the muscle-serum, is faintly acid. The proteid which forms the clot has been called myosin. Besides this another albuminous body, musculin or paramyosinogen (HALLIBURTON), is found in the clot.

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[1] Zeitschr. f. physiol. Chem., Bd. 7.

[2] Upsala Läkaref. Förh., Bd. 28, and Maly's Jahresber., Bd. 23.

[3] Untersuchungen über das Protoplasma. Leipzig, 1864.

Myosin was first discovered by KÜHNE, and constitutes the principal mass of the proteids of the dead muscle, and according to a few investigators it forms the greatest part of the contractile protoplasm. The statements as to the occurrence of myosin in other organs besides the muscles require further proof. The quantity of myosin in the muscles of different animals varies, according to DANILEWSKY,[2] between 30 and 110 p. m.

Myosin is a globulin whose elementary composition, according to CHITTENDEN and CUMMINS,[3] is, on an average, the following: C 52.82, H 7.11, N 16.17, S 1.27, O 22.03%. If the myosin separates as fibres, or if a myosin solution with a minimum quantity of alkali is allowed to evaporate on a microscope-slide to a gelatinous mass, doubly refracting myosin may be obtained. Myosin has the general properties of the globulins. It is insoluble in water, but soluble in dilute saline solutions as well as dilute acids or alkalies. It is completely precipitated by saturating with NaCl, also by $MgSO_4$, in a solution containing 94% of the salt with its water of crystallization (HALLIBURTON[4]). Like fibrinogen it coagulates at + 56° C. in a solution containing common salt, but differs from it since under no circumstances can it be converted into fibrin. The coagulation temperature, according to CHITTENDEN and CUMMINS, not only varies for myosin of different origin, but also for the same myosin in different salt solutions.

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[1] Journal of Physiol., Vol. 8.

[2] Zeitschr. f. physiol. Chem., Bd. 7.

[3] Studies from the Physiol. Laboratory of Yale College, New Haven, vol. 8, p. 115.

[4] Journal of Physiol., Vol. 8.

Myosin may be prepared in the following way, as suggested by HALLIBURTON: The muscle is first extracted by a 5% magnesium, sulphate solution. The filtered extract is then treated with magnesium sulphate in substance until 100 c. c. of the liquid contains about 50 grms. of the salt. The so-called paramyosinogen or musculin separates. The filtered liquid is then treated with magnesium sulphate until each 100 c. c. of the liquid holds 94 grms. of the salt in solution. The myosin which now separates is filtered off, dissolved in water by aid of the retained salt, precipitated by diluting with water, and, when necessary, purified by redissolving in dilute-salt solution and precipitating with water.

The older and perhaps the usual method of preparation consists, according to DANILEWSKY,[1] in extracting the muscle with a 5–10% ammonium-chloride solution, precipitating the myosin from the filtrate by strongly diluting with water, redissolving the precipitate in ammonium-chloride solution, and the myosin obtained from this solution is either reprecipitated by diluting with water or by removing the salt by dialysis.

As the coagulation of the blood-plasma is considered by most investigators as an enzymotic process, so certain observations seem to show that the coagulation of the muscle-plasma is an analogous process. From muscles which had been kept for a long time in alcohol HALLIBURTON obtained, by extracting the mass with water, a soluble substance contaminated with albumose which, although not identical with fibrin-ferment, had the property of accelerating the coagulation of the muscle-plasma. This substance he called "*myosin-ferment.*" It has been shown by the investigations of CAVAZZANI,[2] that the lime-salts are of importance in the coagulation of the muscle-plasma as well as in that of the blood.

As in the blood-plasma we have a mother-substance of fibrin, fibrinogen, so also it is considered that in the muscle-plasma we have a mother-substance of myosin, myosinogen. This body has not thus far been isolated with certainty. HALLIBURTON found that a solution of purified myosin in dilute-salt solution (5% $MgSO_4$), and sufficiently diluted with water, coagulates after a certain time, and at the same time becomes acid and a typical myosin-clot separates. This coagulation, which is accelerated by warming or by the addition of myosin-ferment, is, according to HALLIBURTON, a process analogous to the coagulation of the muscle-plasma. According to this same investigator, myosin when dissolved in water by the aid of a neutral salt is reconverted into myosinogen, while after diluting with water myosin is again produced from the myosinogen. These observations may, however, be explained in other ways. In these cases the separation of the myosin is evidently closely connected with the liquid becoming acid, while the separation of myosin from the muscle-plasma, at least from the muscle-plasma of the frog, is independent of this acidity, for it may take place before the liquid becomes acid. The mother-substance of myosin and the chemical processes of the myosin coagulation are questions which must not be considered as settled.

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[1] Zeitschr. f. physiol. Chem., Bd. 5, S. 158.

[2] Maly's Jahresber., Bd. 22, S. 333.

Musculin, called PARAMYOSINOGEN by HALLIBURTON, is a globulin which is characterized by its low coagulation temperature, about + 47° C., which may vary in different species of animals (+ 45° in frogs, + 51° C. in birds). It is more easily dissolved than myosin by NaCl or $MgSO_4$ (salt containing 50% water of crystallization). Musculin is separated simultaneously with myosin in the coagulation of the muscle-plasma, and it is therefore found in the clot. A solution which contains musculin and no myosin does not coagulate on the addition of the myosin-ferment (HALLIBURTON). If the dead muscle is extracted with water, the musculin passes in part into solution. The musculin may be isolated by fractional precipitation with magnesium sulphate (50 grms. to each 100 c. c. liquid), and may be identified by its low coagulation temperature.

Myoglobulin. After the separation of the musculin and the
n from the salt extract of the muscle by means of $MgSO_4$ the
bulin may be precipitated by saturating the filtrate with
t. It is similar to serglobulin, but coagulates at + 63° C.
BURTON). *Myoalbumin* or muscle-albumin, seems to be
al with seralbumin (seralbumin α, according to HALLIBUR-
and is prepared according to the same method. *Myoalbumose*
albumose) is found in small quantities in the muscles, and
ned by extracting with water the finely divided mass
ich has previously been coagulated by keeping in
ng time (HALLIBURTON).

After the complete removal from the muscle of all proteid bodies which are soluble in water and ammonium chloride, DANILEWSKY[1] claims that an insoluble proteid remains which only swells in ammonium-chloride solution and which forms with the other insoluble constituents of the muscular fibre the "*muscle-stroma.*" According to DANILEWSKY, the amount of such stroma substance is connected with the muscle activity. He maintains that the muscles contain a greater amount of this substance, compared with the myosin present, when the muscles are quickly contracted and relaxed.

According to J. HOLMGREN[2] this stroma substance does not belong to either the nucleoalbumin or the nucleoproteid group. It is not a glycoproteid, as it does not yield a reducing substance when boiled with dilute mineral acids. It is very similar to coagulated proteids and dissolves in dilute alkalies, forming an albuminate. The elementary composition of this substance is nearly the same as that of myosin.

Muscle-syntonin, which may be obtained by extracting the muscles with hydrochloric acid of 1 p. m., and which, according to K. MÖRNER, is less soluble and has a greater aptitude to precipitate than other acid albumins, seems not to occur preformed in the muscles.

Muscle-pigments. There is no question that the red color of the muscles even when completely freed from blood depends in part on hæmoglobin, though it is contested by many. MACMUNN[3] claims that the muscles contain also another coloring substance which is closely allied to the blood-pigments and whose spectrum is very similar to that of hæmochromogen. This coloring matter has been called *myohæmatin.* According to LEVY[4] this myohæmatin is nothing but hæmochromogen, which is produced from oxyhæmoglobin by decomposition and reduction. Nevertheless MACMUNN[5] still adheres to his view that myohæmatin is an independent coloring substance, and in support of his opinion he adduces the fact that myohæmatin is found also in the muscles of insects in which no hæmoglobin occurs.

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[1] Zeitschr. f. Physiol., Bd. 7.

[2] Upsala Läkaref. Förh., Bd. 28, and Maly's Jahresber., Bd. 23.

[3] Phil. Trans. of the Roy. Soc., 1886, Part 1, and Journal of Physiol., Vol. 8.

[4] Zeitschr. f. physiol. Chem., Bd. 13.

[5] *Ibid.*, Bd. 13, S. 497.

The reddish-yellow coloring matter of the muscles of the salmon has been little studied. Traces of enzymes, such as pepsin and diastatic enzymes, have been found in them. The so-called "myosin-ferment," and probably an enzyme producing lactic-acid fermentation, are also found in these muscles.

Extractive Bodies of the Muscles.

The *nitrogenous extractives* consist chiefly of *creatin*, on an average of 2–4 p. m., in the fresh muscles containing water, also the xanthin bases, *hypoxanthin* and *xanthin*, besides *guanin* and *carnin*. The average quantities of hypoxanthin, xanthin, and guanin in 1000 parts of the dried substance of the muscles of oxen are, according to KOSSEL,[1] respectively 2.30, 0.53, and 0.20 grms., and in the embryonic ox-muscles respectively 3.59, 1.11, and 4.12 grms.

Besides these we must also consider as an extractive body the syrupy *inosinic acid* ($C_{10}H_{14}N_4O_{11}$), of which only traces are found in the muscles of certain animals This acid was first prepared by LIEBIG,[2] but not closely studied. LIMPRICHT[3] has found another in the flesh of certain cyprindea, namely, the nitrogenized *protic acid*, and SIEGFRIED[4] has recently found another acid, *carnic acid*, which will be treated of below. *Uric acid*, *urea*, *taurin*, and *leucin* are found as traces in the muscles in certain cases only, of a few species of animals. In regard to the amount of these different extractives in the muscles, KRUKENBERG and WAGNER[5] have shown that it varies greatly in different animals. A large quantity of urea is found in the muscles of the shark and ray; uric acid is found in alligators; taurin in cephalopoda; *glycocoll* in mollusks, pecten irradians; and *creatinin* in luvarus imperialis, etc., etc. The statements are very contradictory in regard to the occurrence of urea in the muscles of higher animals. According to the recent investigations of KAUFMANN[6] urea is a regular constituent of the muscles, the quantity in fresh muscles containing water being 0.27-0 7 p. m.

The xanthin bases, with the exception of carnin, have been treated on pages 102–108, and therefore among the extractive bodies we will first consider the creatin.

Creatin, $C_4H_9N_3O_2 + H_2O$ or METHYLGUANIDIN-ACETIC ACID, $NH:C(NH_2).N(CH_3).CH_2.COOH + H_2O$, occurs in the muscles of vertebrate animals in variable amounts in different species; the largest quantity is found in birds. It is also found in the brain, blood, transudations, and the amniotic fluid. Creatin may be prepared synthetically from cyanamid and sarcosin (methylglycocoll). On boiling with baryta-water it decomposes, with the addition of water, and yields urea, sarcosin, and certain other products.

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[1] Zeitschr. f. physiol. Chem. Bd. 8, S. 408.

[2] Annal. d. Chem. u. Pharm., Bd. 62 (1847).

[3] *Ibid.*, Bd. 127.

[4] Ber. d. k. sächs. Gesellsch. d. Wiss., Math.-phys. Klasse, 1893

[5] Zeitschr. f. Biologie, Bd. 21.

[6] Arch. de Physiol., (5) Tome 6.

Because of this behavior several investigators consider creatin as a step in the formation of urea in the organism. On boiling with acids creatin is easily converted, with the elimination of water, into creatinin, $C_4H_7N_3O$, which occurs in urine, and which has also been found in the muscles of the dog by MONARI[1] (see Chapter XV).

According to ST. JOHNSON[2] no creatin occurs in the fresh flesh of oxen, but a creatinin, differing from that found in urine. Muscle-creatin is produced therefrom by bacterial action.

Creatin crystallizes in hard, colorless, monoclinic prisms which lose their water of crystallization at 100° C. It dissolves in 74 parts of water at the ordinary temperature and 9410 parts absolute alcohol. It dissolves more easily with the aid of heat. Its watery solution has a neutral reaction. Creatin is not dissolved by ether. If a creatin solution is boiled with precipitated mercuric oxide, this is reduced, especially in the presence of alkali, to mercury and oxalic acid, and the disgusting-smelling methyluramin (methylguanidin) is developed. A solution of creatin in water is not precipitated by basic lead acetate, but gives a white, flaky precipitate with mercurous nitrate if the acid reaction is neutralized. When boiled for an hour with dilute hydrochloric acid creatin is converted into creatinin, and may be identified by its reactions.

The preparation and detection of creatin is best performed by the following method of NEUBAUER,[3] which was first used in the preparation of creatin from muscles: Finely cut flesh is extracted with an equal weight of water at + 55° to 60° C. for 10–15 minutes, pressed and extracted again with water. The proteids are removed from the united extracts as far as possible by coagulation at boiling heat, the filtrate precipitated by the careful addition of basic lead acetate, the lead removed from this filtrate by H_2S and carefully concentrated to a small volume. The creatin, which crystallizes in a few days, is collected on a filter, washed with alcohol of 88%, and purified, when necessary, by recrystallization. The quantitative estimation of creatin is performed according to the same method.

Carnin, $C_7H_8N_4O_3 + H_2O$, is one of the substances found by WEIDEL[4] in American meat extract. It has also been found by KRUKENBERG and WAGNER[5] in frog-muscles and in the flesh of fishes, and by POUCHET[1] in the urine. Carnin may be transformed into hypoxanthin by oxidation.

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[1] Maly's Jahresber., Bd. 19, S. 296.

[2] Proc. Roy. Soc. Cited from Maly's Jahresber., Bd. 22.

[3] Zeitschr. f. anal. Chem., Bdd. 2 and 6.

[4] Annal. d. Chem. u. Pharm., Bd. 158.

[5] Sitzungsber. d. Würz. phys.-med. Gesellsch., 1883.

Carnin has been obtained as a white crystalline mass. It dissolves with difficulty in cold water, but dissolves easily in warm. It is insoluble in alcohol and ether. It dissolves in warm hydrochloric acid and yields a salt, crystallizing in shining needles, which gives a double combination with platinum chloride. Its watery solution is precipitated by silver nitrate, but this precipitate is neither dissolved by ammonia nor by warm nitric acid. Carnin does not give the so-called WEIDEL's xanthin reaction. Its watery solution is precipitated by basic lead acetate; still the lead combinations may be dissolved on boiling.

Carnin is prepared by the following method: The meat extract diluted with water is completely precipitated by baryta-water. The filtrate is precipitated by basic lead acetate, the lead precipitate boiled with water, filtered while hot, and sulphuretted hydrogen passed through the filtrate. Remove the lead sulphide from the filtrate and concentrate strongly. The concentrated solution is now completely precipitated with silver nitrate, the precipitate washed free from silver chloride by ammonia, and the carnin silver oxide suspended in water and treated with sulphuretted hydrogen.

Carnic Acid is the name given by SIEGFRIED[2] to the acid isolated by him from meat extract and from the watery extract of the muscles. It has the formula $C_{10}H_{15}N_3O_5$. It is readily soluble in water, and its warm alcoholic solution deposits undefined crystalline surfaces on cooling. It gives several crystalline salts, amongst which the silver salt, with 42.6% silver, is of the greatest importance. Carnic acid gives the biuret test, but not Millon's reaction, and it is so very similar to antipeptone (from which it differs by not having sulphur in the molecule) that SIEGFRIED considers it identical therewith. Sulphuretted hydrogen is oxidized by carnic acid in the presence of air into thiosulphuric acid; with hydrochloric acid it yields, by addition, a very solid combination, and with phosphoric acid it forms an acid, phosphocarnic acid. This last-mentioned acid forms soluble salts with calcium and magnesium, and SIEGFRIED considers carnic acid as a substance which simultaneously transports phosphoric acid, lime, magnesia, and also iron in the organism. Phosphocarnic acid gives also a combination with iron, which is soluble in alkalies and alkali carbonates. SIEGFRIED calls such a combination *carniferrin*. Carnic acid occurs in muscle extracts as phosphocarnic acid; but as no true peptone has been heretofore detected in fresh muscles, it is a question whether carnic acid is a physiological constituent of muscles or only a laboration product. According to SIEGFRIED[3] phosphocarnic acid yields carnic acid (antipeptone),

[1] Cited from Neubauer-Hüppert, Analyse des Harns, 10. Aufl., S. 335

[2] Du Bois-Reymond's Arch., Physiol. Abth., 1894.

[3] Ber. d. deutsch. chem. Gesellsch., Bd. 28.

phosphoric acid, and a carbohydrate as cleavage products. It therefore stands in close relationship to the nucleins, and SIEGFRIED suggests the name *paranucleon*, to show that on cleavage they yield a peptone substance, and not proteid like the paranucleins.

Carnic acid is best prepared by precipitating the extract, which has been freed from proteid, by baryta-water at the ordinary temperature, being careful not to add an excess. The filtrate contains the barium salt of the phosphocarnic acid, which is precipitated as carniferrin by ferric chloride at the boiling temperature. This carniferrin is decomposed by barium hydrate at 50° C. The excess of barium is removed from the filtrate by sulphuric acid, filtered, concentrated, and the carnic acid precipitated by alcohol. The acid is purified by repeated precipitation with alcohol.

We must also include among the nitrogenous extractives those bodies which were first discovered by GAUTIER[1] and which occur only in very small quantities, namely, the leucomaines, *xanthocreatinin*, $C_5H_{10}N_4O$, *crusocreatinin*, $C_5H_8N_4O$, *amphicreatinin*, $C_9H_{19}N_7O_4$, and *pseudoxanthin*, $C_4H_5N_5O$.

In the analysis of meat and for the detection and separation of the various extractive bodies of the same we make use of the systematic method as suggested by GAUTIER,[2] for details of which we must refer the reader to the original article.

The **non-nitrogenous extractive bodies** of the muscles are *inosit*, *glycogen*, *dextrose*, and *lactic acid*.

Inosit, $C_6H_{12}O_6 + H_2O$. This body, discovered by SCHERER, is not a carbohydrate, but belongs to the aromatic series and seems to be hexahydroxybenzol (MAQUENNE[3]). With hydriodic acid it yields benzol and tri-iodophenol. Inosit is found in the muscles, liver, spleen, kidneys, suprarenal cavity, lungs, brain, testicles, and in the urine in pathological cases, and as traces in normal urine. It is found very widely distributed in the vegetable kingdom, especially in unripe fruits and in green beans (*phaseolus vulgaris*), and therefore it is also called PHASEOMANNIT.

Inosit crystallizes in large, colorless, rhombic crystals of the monoclinic system, or, if not pure and if only a small quantity crystallizes, it forms groups of fine crystals similar to cauliflower. It loses its water of crystallization at 110° C., also if exposed to the air for a long time. Such exposed crystals are non-transparent and milk-white. The crystals melt at 217° C. Inosit dissolves in 7.5

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[1] Maly's Jahresber., Bd. 16, S. 523.

[2] *Ibid.*, Bd. 22, S. 335.

[3] Bull. de la Soc. chim. (2), Tome 47 and 48; Comp. rend., Tome 104.

parts of water at ordinary temperature, and the solution has a sweetish taste. It is insoluble in strong alcohol and in ether. It dissolves copper oxyhydrate in alkaline solutions, but does not reduce on boiling. It gives negative results with MOORE's test and with BÖTTGER-ALMEN's bismuth test. It does not ferment with beer-yeast, but may undergo lactic- and butyric-acid fermentation. The lactic acid formed thereby is sarcolactic acid according to HILGER,[1] and fermentation lactic acid according to VOHL.[2] Inosit is oxidized into rhodizonic acid by an excess of nitric acid, and the following reactions depend upon this behavior:

If inosit is evaporated to dryness on platinum-foil with nitric acid and the residue treated with ammonia and a drop of calcium-chloride solution, and carefully re-evaporated to dryness, a beautiful rose-red residue is obtained (SCHERER's inosit test). If we evaporate an inosit solution to incipient dryness and moisten the residue with a little mercuric-nitrate solution, we obtain a yellowish residue on drying, which becomes a beautiful red on strongly heating. The coloration disappears on cooling, but it reappears on gently warming (GALLOIS's inosit test).

To prepare inosit from a liquid or from a watery extract of a tissue, the proteids are first removed by coagulating at boiling heat. The filtrate is precipitated by sugar of lead, this filtrate boiled with basic lead acetate and allowed to stand 24–48 hours. The precipitate thus obtained, which contains all the inosit, is decomposed in water by H_2S. The filtrate is strongly concentrated, treated with 2–4 vols. hot alcohol, and the liquid removed as soon as possible from the tough or flaky masses which ordinarily separate. If no crystals separate from the liquid within 24 hours, then treat with ether until the liquid has a milky appearance and allow it to stand. In the presence of a sufficient quantity of ether, crystals of inosit separate within 24 hours. The crystals thus obtained, as also those which are obtained from the alcoholic solution directly, are recrystallized by redissolving in very little boiling water and the addition of 3–4 vols. alcohol.

Glycogen is a constant constituent of the living muscle, while it may be absent in the dead muscle. The quantity of glycogen varies in the different muscles of the same animal. BÖHM[3] found 10 p. m. glycogen in the muscles of cats, and moreover he found a greater amount in the muscles of the extremities than in those of the rump. The food also has a great influence. BÖHM found 1–4 p. m. glycogen in the muscles of fasting animals and 7–10 p. m. after partaking of food. LUCHSINGER maintains an opinion, formerly generally accepted, that in starvation, or if there is a lack of carbohydrates in the food, glycogen disappears more quickly from the muscles than from the liver; but according to ALDEHOFF exactly the reverse takes place. The glycogen disappears more quickly in starvation from the liver than from the muscles, not only in hens, as observed by WEISS, but also in other animals, such as the pigeon, rabbit, cat, and horse.[1]

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[1] Annal. d. Chem. u. Pharm., Bd. 160.

[2] Ber. d. deutsch. chem. Gesellsch., Bd. 9.

[3] Pflüger's Arch., Bd. 23, S. 44.

Muscle-sugar, of which traces only occur in the living muscle and which is probably formed after the death of the muscle from the muscle-glycogen, is, according to the investigations of PANORMOFF,[2] probably dextrose. As an intermediate step in this sugar-formation we must mention dextrin, which is sometimes found in the muscles. Perhaps this dextrin has been confounded with glycogen.

Lactic Acids. Of the oxypropionic acids with the formula $C_3H_6O_3$ there is one, hydracrylic acid, $CH_2(OH).CH_2.COOH$, which is not found in the animal body and therefore has no physiological chemical interest. Indeed only α-oxypropionic acid or ethylidene lactic acid, $CH_3.CH(OH).COOH$, of which we have three physical isomers, is of importance. These three ethylidene lactic acids are the ordinary, optically inactive FERMENTATION LACTIC ACID, the dextrorotatory PARALACTIC or SARCOLACTIC ACID, and the LÆVOLACTIC ACID obtained by SCHARDINGER[3] by the fermentation of cane-sugar by means of a special bacillus. This lævolactic acid has also been detected by BLACHSTEIN[4] in the culture of GAFFKY's typhoid bacillus in a solution of sugar and peptone.

The *fermentation lactic acid*, which is formed from milk-sugar by allowing milk to sour and by the acid fermentation of other carbohydrates, is considered to exist in small quantities in the muscles (HEINTZ[5]), in the gray matter of the brain (GSCHEIDLEN[6]), and in diabetic urine. During digestion this acid is also found in the contents of the stomach and intestine, and as alkali lactate in the chyle. The *paralactic acid* is, at all events, the true acid of meat extracts, and this alone has been found with certainty in dead muscle. The lactic acid which is found in the spleen, lymphatic glands, thymus, thyroid gland, blood, bile, pathological transudations, osteomalacious bones, in perspiration in puerperal fever, and in the urine after fatiguing marches, in acute yellow atrophy of the liver, in poisoning by phosphorus, especially after extirpation of the liver (in geese according to MINKOWSKI,[1] in frogs according to MARCUSE[2] and WERTHER[3]), seems to be paralactic acid.

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[1] See Chapter VIII, p. 211, and references to the literature of Glycogen in the above chapter.

[2] Zeitschr. f. physiol. Chem., Bd. 17.

[3] Monatshefte f. Chem., Bd. 11.

[4] Arch. des sciences biol. de St. Pétersbourg, Tome 1, p. 199.

[5] Annal. d. Chem. u. Pharm , Bd. 157.

[6] Pflüger's Arch., Bd. 8, S. 171.

The origin of paralactic acid in the animal organism has been sought by several investigators, who took for basis the researches of GAGLIO,[4] MINKOWSKI,[5] and ARAKI,[6] in a decomposition of proteid in the tissues. GAGLIO claims a lactic-acid formation by passing blood through the kidneys and lungs. He also found 0.3–0.5 p. m. lactic acid in the blood of a dog after proteid food and only 0.17–0.21 p. m. after fasting for 48 hours. According to MINKOWSKI the quantity of lactic acid eliminated by the urine in animals with extirpated livers is increased with proteid food, while the administration of carbohydrates has no effect. ARAKI has also shown that if we produce a scarcity of oxygen in animals (dogs, rabbits, and hens) by poisoning with carbon monoxide, by the inhalation of air deficient in oxygen, or by any other means, a considerable elimination of lactic acid (besides dextrose and also often albumin) takes place by the urine. As a scarcity of oxygen, according to the ordinary statements, produces an increase of the proteid katabolism in the body, the increased elimination of lactic acid in these cases must be due in part to an increased proteid destruction and in part to a diminished oxidation.

ARAKI has not drawn such a conclusion from his experiments, but he considers the abundant formation of lactic acid to be due to a cleavage of the sugar formed from the glycogen. He found that in all cases where lactic acid and sugar appeared in the urine the quantity of glycogen in the liver and muscles was always diminished. He also calls attention to the fact that dextrolactic acid may be formed from glycogen, as directly observed by Ekunina,[1] and also to the numerous observations on the formation of lactic acid and the consumption of glycogen in muscular activity. Without denying the possibility of a formation of lactic acid from proteid, he states that with lack of oxygen we have to deal with an incomplete combustion of the lactic acid derived by a cleavage of the sugar. Hoppe-Seyler[2] also positively defends the view as to the formation of lactic acid from carbohydrates. He is of the view that lactic acid is produced from the carbohydrates by the cleavage of the sugar only with lack of oxygen, while with sufficient oxygen the sugar is burnt into carbon dioxide and water. The formation of lactic acid in the absence of free oxygen and in the presence of glycogen or dextrose is, according to Hoppe-Seyler, very probably a function of all living protoplasm. We have good ground for the assumption as to the formation of lactic acid from proteid as well as from carbohydrates.

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[1] Arch. f. exp. Path. u. Pharm., Bd. 21, S. 41.

[2] Pflüger's Arch., Bd. 39.

[3] *Ibid.*, Bd. 46.

[4] Du Bois-Reymond's Arch., 1886.

[5] Arch. f. exp. Path. u. Pharm., Bd. 21.

[6] Zeitschr. f. physiol. Chem., Bdd. 15, 16, 17, and 19.

The lactic acids are amorphous. They have the appearance of colorless or faintly yellowish, acid-reacting syrups which mix in all proportions with water, alcohol, or ether. The salts are soluble in water, and most of them also in alcohol. The two acids are differentiated from each other by their different optical properties —paralactic acid being dextrogyrate, while fermentation lactic acid is optically inactive—also by their different solubilities and the different amounts of water of crystallization of the calcium and zinc salts. The zinc salt of fermentation lactic acid dissolves in 58–63 parts of water at 14–15° C. and contains 18.18% water of crystallization, corresponding to the formula $Zn(C_3H_5O_3)_2 + 3H_2O$. The zinc salt of paralactic acid dissolves in 17.5 parts of water at the above temperature and contains ordinarily 12.9% water, corresponding to the formula $Zn(C_3H_5O_3)_2 + 2H_2O$. The calcium salt of fermentation lactic acid dissolves in 9.5 parts water and contains 29.22% (= 5 mol.) water of crystallization, while calcium paralactate dissolves in 12.4 parts water and contains 24.83 or 26.21% (= 4 or 4½ mol.) water of crystallization. Both calcium salts crystallize, not unlike tyrosin, in spheres or tufts of very fine microscopic needles.

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[1] Journal f. prakt. Chem. (N. F.), Bd. 20.

[2] Virchow's Festschrift, also Ber. d. deutsch. chem. Gesellsch., Bd. 25. Referatb., S. 685.

HOPPE-SEYLER and ARAKI,[1] who have closely studied the optical properties of the lactic acids and lactates, consider the lithium salt as best suited for the preparation and quantitative estimation of the lactic acids. The lithium salt contains 7.29% Li. They are readily soluble in water and crystallize pure and anhydrous very easily from boiling alcohol.

Lactic acids may be detected in organs and tissues in the following manner: After complete extraction with water the proteid is removed by coagulation at boiling temperature and the addition of a small quantity of sulphuric acid. The liquid is then exactly neutralized while boiling with caustic baryta, and then evaporated to a syrup after filtration. The residue is precipitated with absolute alcohol, and the precipitate completely extracted with alcohol. The alcohol is entirely distilled from the united alcoholic extracts, and the neutral residue is shaken with ether to remove the fat. The residue is dissolved in water and phosphoric acid added, and repeatedly shaken with fresh quantities of ether, which dissolves the lactic acid. The ether is now distilled from the several ethereal extracts, the residue dissolved in water, and this solution carefully warmed on the water-bath to remove the last traces of ether and volatile acids. A solution of zinc lactate is prepared from this filtered solution by boiling with zinc carbonate, and this is evaporated until crystallization commences and then allowed to stand over sulphuric acid. An analysis of the salts is necessary in careful work.

Fat is never absent in the muscles. Some fat is always found in the intermuscular connective tissue; but the muscle-fibres themselves also contain fat. The quantity of fat in the real muscle substance is always small, usually amounting to about 10 p. m. or somewhat more. A considerable quantity of fat in the muscle-fibres is only found in fatty degeneration. *Lecithin* is also habitually found in the muscles.

The *Mineral Bodies of the Muscles.* We have no complete analyses of the mineral substances of the pure, blood-free muscle substance. The ash remaining after burning the muscle, which amounts to about 10–15 p. m., calculated on the moist muscle, is acid in reaction. The largest constituents are potassium and phosphoric acid. Next in amount we have sodium and magnesium, and lastly calcium, chlorine, and iron oxide. Sulphates only exist as traces in the muscles, but are formed by the burning of the proteids of the muscles, and therefore occur in abundant quantities in the ash. The muscles contain such a large quantity of potassium and phosphoric acid that potassium phosphate seems to be unquestionably the predominating salt. Chlorine is found in such insignificant quantities that it is perhaps derived from a contamination with blood or lymph. The quantity of magnesium is about double that of calcium. These two bodies, as well as iron, occur only in very small amounts.

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[1] Zeitschr. f. physiol. Chem., Bd. 20.

The *gases* of the muscles consist of large quantities of carbon dioxide, besides traces of nitrogen.

Rigor Mortis of the Muscles. If the influence of the circulating oxygenated blood is removed from the muscles, as after death of the animal or by ligature of the aorta or the muscle-arteries (STENSON'S test), *rigor mortis* sooner or later takes place. The ordinary rigor appearing under these circumstances is called the spontaneous or the fermentive rigor, because it seems to depend in part on the action of an enzyme. A muscle may also become stiff for other reasons. The muscles may become momentarily stiff by warming, in the case of frogs to 40°, in mammalia to 48–50°, and in birds to 53° C. (heat-rigor). Distilled water may also produce a rigor in the muscles (water-rigor). Acids even when very weak, such as carbon dioxide, may quickly produce a rigor (acid-rigor), or hasten its appearance. A number of chemically different substances, such as chloroform, ether, alcohol, ethereal oils, caffein, and many alkaloids, produce a similar effect. The rigor which is produced by means of acids or other agents which, like alcohol, coagulate proteids must be considered as produced by entirely different processes from those causing spontaneous rigor.

The time within which the spontaneous rigor occurs depends upon the temperature; a low temperature retarding and a high temperature hastening its appearance. Muscular activity also exercises an appreciable influence on the rigor of the muscles, for a previous active contraction accelerates the rigor of the muscles; the mechanical abuse of the muscles of various kinds operates in the same way. The appearance of spontaneous rigor is under the influence of the central nervous system, and a muscle whose nerve has been severed stiffens more slowly than one whose continuity with the central nervous system has not been destroyed (HERMANN and his pupils v. EISELBERG,[1] v. GENDRE,[2] and BIERFREUND[3]). The nervous system seems also to have a similar influence on the *post-mortem* acidification of the muscles (GROSS[1]). HERMANN and his pupils[2] consider the *rigor mortis* as a final slowly proceeding muscle-contraction identical with the ordinary contraction. GOTSCHLICH[3] has indeed made the statement that rest, activity, and rigor of the muscles are identical processes in principle. This cannot at present be positively proven from a chemical standpoint.

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[1] Pflüger's Arch., Bd. 24.

[2] *Ibid.*, Bd. 35, S. 45.

[3] *Ibid.*, Bd. 43.

When the muscle passes into *rigor mortis* it becomes shorter and thicker, harder and non-transparent, less ductile. The acid part of the amphoteric reaction becomes stronger, which is explained by most investigators by a formation of lactic acid. There is hardly any doubt that this increase in acidity may at least in part be due to a transformation of a part of the diphosphate into monophosphate by the lactic acid. The statements in regard to the occurrence also of free lactic acid or not in the rigor mortis muscle are contradictory.[4] The chemical processes which take place in rigor of the muscles, besides the formation of acid, are the following: By the coagulation of the plasma a myosin-clot is produced which is the cause of the hardening and of the diminished transparency of the muscle. The appearance of this clot may be hastened by the simultaneous occurrence of lactic acid. Carbon dioxide is also formed, which does not seem to be a direct oxidation product, but a product of the cleavage processes. HERMANN[5] claims that carbon dioxide is produced in the removed muscle, even in the absence of oxygen, when it passes into rigor mortis.

As many investigators admit of an increased formation of lactic acid on the appearance of rigor mortis, the question arises, from what constituents of the muscle is this acid derived? The most probable explanation is that the lactic acid is produced from the glycogen, as certain investigators, such as NASSE[6] and WERTHER,[7] have observed a decrease in the quantity of glycogen in rigor of the muscle. On the other side, Böhm[1] has observed cases in which no consumption of glycogen took place in rigor of the muscle, and he has also found that the quantity of lactic acid produced is not proportional to the quantity of glycogen. It is therefore possible that the consumption of glycogen and the formation of lactic acid in the muscles are two processes independent of each other, and, as above stated in regard to the formation of paralactic acid, the lactic acid of the muscle may be considered as a decomposition product of proteid. The origin of the carbon dioxide is also not to be sought for in the decomposition of the glycogen or dextrose. Pflüger and Stintzing[2] have found that in the muscle a substance occurs which evolves large quantities of carbon dioxide on boiling with water, and it is probably this substance which is decomposed with the formation of carbon dioxide in tetanus as well as in rigor. Tissot[3] has observed a true respiration in removed muscle, which is independent of the putrefactive processes, and by which oxygen is absorbed and carbon dioxide eliminated, this being contrary to Hermann's assertion. The carbon dioxide eliminated originates from two sources. A part is preformed in the muscle and is only physically evolved carbon dioxide, and another part is formed in the removed muscle.

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[1] Centralbl. f. Physiol., Bd. 2, S. 91.

[2] See Bierfreund, l. c.

[3] Pflüger's Arch., Bd. 56.

[4] It is impossible to enter into details of the disputed statements as to the reaction of the muscles, etc. We will only refer to the works of Röhmann, Pflüger's Arch., Bdd. 50 and 55, and Hefter, Arch. f. exp. Path. u. Pharm., Bd. 31.

[5] Untersuchungen über den Stoffwechsel der Muskeln, etc. Berlin, 1867.

[6] Beitr. z. Physiol. der Kontraktil. Substanz, Pflüger's Arch., Bd. 2.

[7] Pflüger's Arch., Bd. 46.

After the muscles have been rigid for some time they relax again and the muscles become softer. This is in part produced by the strong acid dissolving the myosin clot and in part, and in all probability mainly, upon the commencement of putrefaction.

Metabolism in the Inactive and Active Muscles. It is admitted by a number of prominent investigators, Pflüger and Colasanti,[4] Zuntz and Röhrig,[5] and others, that the exchange of material in the muscles is regulated by the nervous system. When at rest, when there is no mechanical exertion, we have a condition which Zuntz and Röhrig have designated "*chemical tonus*." This tonus seems to be a reflex tonus, for it may be reduced by discontinuing the connection between the muscles and the central organ of the nervous system by cutting through the spinal cord or the muscle-nerves, or by paralyzing the same by means of curara poison. It may also be reduced or checked by adjusting the temperature between the skin and the surrounding medium; or it may be increased by the reverse, by irritating the nerves of the skin by cooling. The possibility of reducing the chemical tonus of the muscles by any of the above-mentioned means, but especially by the action of curara, offers an important means of deciding the extent and kind of chemical processes going on in the muscles when at rest. In comparative chemical investigation of the processes in the active and the inactive muscles several methods of procedure have been adopted. The removed homologous, active and inactive muscles have been compared, also the arterial and venous muscle-blood in rest and activity, and lastly the total exchange of material, the receipts and expenditures of the organism, have been investigated under these two conditions.

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[1] Pflüger's Arch., Bdd. 23 and 46.

[2] *Ibid.*, Bd. 18.

[3] Arch. de Physiol., Sér. 5, Tome 7.

[4] See the works of Pflüger and his pupils in Pflüger's Arch., Bdd. 4, 12, 14, 16, 18.

[5] *Ibid.*, Bd. 4, S. 57; also Zuntz., *ibid.*, Bd. 12, S. 522.

By investigations according to these several methods it has been found that the active muscle takes up oxygen from the blood and returns to it carbon dioxide, and also that the quantity of oxygen taken up is greater than the oxygen contained in the carbon dioxide eliminated at the same time. The muscle, therefore, holds in some form of combination a part of the oxygen taken up while at rest. During activity the exchange of material in the muscle, and therewith the exchange of gas, is increased. The animal organism takes up considerably more oxygen in activity than when at rest, and eliminates also considerably more carbon dioxide. The quantity of oxygen which leaves the body as carbon dioxide during activity is considerably larger than the quantity of oxygen taken up at the same time; and the venous muscle-blood is poorer in oxygen and richer in carbon dioxide during activity than during rest. The exchange of gases in the muscles during activity is the reverse of that at rest, for the active muscle gives up a quantity of carbon dioxide which does not correspond to the quantity of oxygen taken up, but is considerably greater. It follows from this that in muscular activity not only does oxidation take place, but also splitting processes occur. This follows also from the fact that removed blood-free muscles when placed in an atmosphere devoid of oxygen can labor for some time and also yield carbon dioxide (HERMANN[1]).

During muscular inactivity, in the ordinary sense, a consumption of glycogen takes place. This is inferred from the observations of several investigators that the quantity of glycogen is increased and its corresponding consumption reduced in those muscles whose chemical tonus is reduced either by cutting through the nerve or for other reasons (BERNARD,[1] CHANDELON,[2] WAY,[3] and others). In activity this consumption of glycogen is increased, and it has been positively proved by the researches of several investigators (NASSE,[4] WEISS,[5] KÜLZ,[6] MARCUSE,[7] MANCHÉ,[8] MORAT and DUFOUR[9]) that the quantity of glycogen in the muscles in activity decreases quickly and freely. By investigating with the muscles *in situ*, especially on the *levator labii superioris* of a horse, CHAUVEAU and KAUFMANN[10] have not only confirmed the above facts in regard to the exchange of gas during rest and activity, but they also found that the muscles remove sugar from the blood, and indeed considerably more during activity than when at rest. They found (calculating the amount found in 1 gramme of muscle per minute to 1 kilo per hour) that 1 kilo of muscle removes 2.186 grms. sugar from the blood per hour during rest, while it removes 8.416 grms. per hour in activity. Strong objections to the conclusions drawn from these experiments have been made by SEEGEN[11]; although these experiments may not be quite conclusive, still it cannot be denied that an increased consumption of sugar takes place during activity. Other investigators, such as QUINQUAUD,[12] MORAT and DUFOUR, have observed a consumption of the sugar derived from the blood during work, and finally in this connection we must recall that SEEGEN[13] and still earlier CHAUVEAU have come to a similar conclusion by special investigations. According to SEEGEN the blood-sugar is on the whole the source of heat and work. SEEGEN[14] has determined the quantity of sugar in the arterial and venous blood of the muscle during rest and when directly or indirectly irritated, but obtained no constant results. He found, on the contrary, generally a very considerable consumption of the glycogen in the active muscles. SEEGEN calculates that in his experiments, with the assumption that the glycogen was completely oxidized, the glycogen in greatest part served as heat-former and only to a small extent, in most cases 5–10% of its store of energy, as mechanical work. The entire quantity of glycogen in the animal body is, according to SEEGEN, only sufficient to supply a small fraction of the mechanical work of the body, and the most important source of mechanical work and of heat lies, according to him, in the blood-sugar.

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[1] L. c.

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[1] Compt. rend., Tome 48, p. 673.

[2] Pflüger's Arch., Bd. 13.

[3] Arch. f. exp. Path. u. Pharm., Bd. 34.

[4] Pflüger's Arch., Bd. 2.

[5] Wien. Sitzungsber., Bd. 64, Abth. 1.

[6] See Külz in Ludwig's Festschrift. Marburg, 1891.

[7] Pflüger's Arch., Bd. 39.

[8] Zeitschr. f. Biologie, Bd. 25.

[9] Arch. de Physiol. (5) Bd. 4.

[10] Compt. rend., Tome 103, 104, and 105.

[11] Centralbl. f. Physiol., Bd. 8, S. 417.

[12] Maly's Jahresber., Bd. 16, S. 321.

[13] Die Zuckerbildung im Thierkörper (Berlin, 1890), and Pflüger's Arch., Bd. 50.

[14] Centralbl. f. Physiol., Bd. 8, and Du Bois-Reymond's Arch., 1895.

The amphoteric reaction of the inactive muscles is changed during activity to an acid reaction (DUBOIS-REYMOND and others), and the acid reaction increases to a certain point with the work. The quickly contracting pale muscles produce, according to GLEISS,[1] more acid during activity than the more slowly contracting red muscles. The acid reaction appearing during activity was formerly considered due to the formation of lactic acid, a view which has been contradicted by ASTASCHEWSKY,[2] PFLÜGER and WARREN,[3] who found less lactic acid in the tetanized muscle than when at rest. MONARI[4] also found a decrease in the quantity of lactic acid during activity, and according to HEFTER[5] the quantity of lactic acid in the muscle is diminished in tetanus produced by poison. Contrary to these investigations MARCUSE[6] and WERTHER[7] have been able to prove the formation of lactic acid during activity; still the statements are very contradictory. Other observations speak for a formation of lactic acid during activity. Thus SPIRO[8] found an increase in the quantity of lactic acid in the blood during work. COLASANTI and MOSCATELLI[9] found small quantities of lactic acid in human urine after strenuous marches, and WERTHER observed abundance of lactic acid in the urine of frogs after tetanization. According to HOPPE-SEYLER,[1] on the contrary, in agreement with his view in regard to the formation of lactic acid, a formation of lactic acid does not take place regularly during work, but only when insufficient oxygen is supplied. ZILLESEN[2] has also found that on artificially cutting off the oxygen from the muscles during life more lactic acid was formed than under normal conditions.

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[1] Pflüger's Arch., Bd. 41.

[2] Zeitschr. f. physiol. Chem., Bd. 4.

[3] Pflüger's Arch., Bd. 24.

[4] Maly's Jahresber., Bd. 19, S. 303.

[5] Arch. f. exp. Path. u. Pharm., Bd. 31.

[6] L. c.

[7] Pflüger's Arch., Bd. 46.

[8] Zeitschr. f. physiol. Chem., Bd. 1.

[9] Maly's Jahresber., Bd. 17, S. 212.

It is evident that the experiments with the muscles *in situ*, in other words with muscles through which blood is passing, cannot yield any conclusion to the above question, as the lactic acid formed during work may perhaps be removed by the blood. The following objections can be made against those experiments in which lactic acid has been found after moderate work in the blood or the urine, as also especially against the experiments with removed active muscles, namely, that in these cases the supply of oxygen to the muscles was not sufficient, and that the lactic acid formed thereby is not, corresponding to the views of HOPPE-SEYLER, a perfectly normal process. The question as to the formation of lactic acid in the active muscle under perfectly physiological conditions is still an open one.

According to WEYL and ZEITLER,[3] the active muscle contains more phosphoric acid (in part formed by the decomposition of lecithin) than the inactive muscle. As in the dead muscle, so in the active muscle, the somewhat stronger acid reaction is in part due to a greater quantity of monophosphate.

The amount of proteids in the removed muscles is, according to the older investigators, decreased by work. The correctness of this statement is, however, disputed by other investigators. Also the older statements in regard to the nitrogenous extractive bodies of the muscle in rest and in activity are uncertain. According to the recent researches of MONARI,[4] the total quantity of creatin and creatinin is increased by work; and indeed the amount of creatinin is especially augmented by an excess of muscular activity. The creatinin is formed essentially from the creatin. In excessive activity MONARI also found xantho-creatinin in the muscle, and the quantity was one tenth of that of the creatinin. The quantity of xanthin bodies is, according to MONARI, decreased under the influence of work. It seems to have been positively shown that the active muscle contains a smaller quantity of bodies soluble in water and a larger quantity of bodies soluble in alcohol than the resting muscle (HELMHOLTZ).[1]

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[1] L. c. and Zeitschr. f. physiol Chem., Bd. 19, S. 476.

[2] Zeitschr. f. physiol. Chem., Bd. 15.

[3] Zeitschr. f. physiol. Chem., Bd. 6, S. 557.

[4] Maly's Jahresber., Bd. 19, S. 296.

An attempt has been made to solve the question relative to the behavior of the nitrogenized constituents of the muscle at rest and during activity by determining the total quantity of nitrogen eliminated under these different conditions of the body. While formerly it was held with LIEBIG that the elimination of nitrogen by the urine was increased by muscular work, the researches of several experimenters, especially those of VOIT[2] on dogs and PETTENKOFER and VOIT[3] on men, have led to quite different results. They have shown, as has also lately been confirmed by other investigators, especially HIRSCHFELD,[4] that during work no increase or only a very insignificant increase in the elimination of nitrogen takes place. We should not omit to mention the fact that a series of experiments has been made showing a significant increase in the metabolism of proteids during or after work. We have as example the observations of FLINT[5] and PAVY[6] on a pedestrian, v. WOLFF, v. FUNKE, KREUZHAGE and KELLNER[7] on a horse, and lately those of ARGUTINSKY[8] and KRUMMACHER[9] on themselves, which show an undoubted increase in the elimination of nitrogen during or after work.

The elimination of nitrogen is mainly dependent upon causes which will be spoken of later (Chapter XVIII), such as the quantity and composition of the food, the condition of the adipose tissue, the action of work on the respiratory mechanism, etc., etc., all of which can hardly have received sufficient consideration in the last-mentioned experiments.[1] The strong proof which the very careful experiments of VOIT, of PETTENKOFER and VOIT, and of HIRSCHFELD furnish in support of this theory is hardly affected by these investigations, though we must admit that this question is still somewhat unsettled. Even if we consider the question that muscular work does not cause any increase in the elimination of nitrogen as quite positively proved, still we do not exclude the possibility of an increased metabolism of proteids in the muscle. It is possible on account of the functional exchange action of the organs, of which RANKE[2] has made a special study, that an increased metabolism of proteid in the muscles may be compensated by a simultaneous decreased metabolism of proteid in other organs. But however this may be, the modern view is, notwithstanding, that the metabolism of proteid in the muscle is not increased by activity.

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[1] Arch. f. Anat. u. Physiol., 1845.

[2] Untersuchungen über den Einfluss des Kochsalzes, des Kaffees und der Muskelbewegungen auf den Stoffwechsel (München, 1860), and Zeitschr. f. Biologie, Bd. 2.

[3] Zeitschr. f. Biologie, Bd. 2.

[4] Virchow's Arch., Bd. 121.

[5] Journal of Anat. and Physiol., Vols. 11 and 12.

[6] The Lancet, 1876 and 1877.

[7] Cited by Voit in Hermann's Handbuch, Bd. 6, S. 197.

[8] Pflüger's Arch., Bd. 46.

[9] *Ibid.*, Bd. 47.

The quantity of metabolic products containing sulphur may also be a measure of the extent of the metabolism of proteids, and this quantity may be determined by estimating the suphur in the urine. An increase in the elimination of sulphur after work has been observed for a long time by ENGELMANN,[3] and also by FLINT and PAVY. As sulphuric acid and also non-oxidized sulphur are eliminated by the urine, it is necessary to determine the total sulphur eliminated during work and after work. BECK and BENEDIKT[4] have made investigations of this kind, and they find that the elimination of sulphur is increased by work and diminished after work, which speaks for an increased proteid metabolism during work. I. MUNK[5] by observations on resting and working persons has given further proof that the elimination of nitrogen and sulphur (also phosphoric acid and potash) runs parallel with the metabolism of proteid. The increased elimination of sulphur was not in the neutral sulphur, but nearly entirely in the oxidized sulphur.

The investigations on the amount of fat in removed muscles during activity and at rest have not led to any definite results. The metabolic experiments of VOIT on a starving dog, and those of

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[1] See Voit in Hermann's Handbuch, Bd. 6, Kap. 3, Abschn., 9; I. Munk, Du Bois-Reymond's Arch., 1890; and Hirschfeld, l. c.

[2] Die Blutvertheilung und der Thätigkeitswechsel der Organe. Leipzig, 1871.

[3] Du Bois-Reymond's Arch., 1871.

[4] Pflüger's Arch., Bd. 54.

[5] Verhandl. d. physiol. Gesellsch. zu Berlin, 1894–95.

PETTENKOFER and VOIT on a man, offer strong proofs to show that an increased decomposition of the fat takes place during activity.

If the results of the investigations thus far made of the chemical processes going on in the active and inactive muscle were collected together, we would find the following characteristics for the active muscle. The active muscle takes up more oxygen and gives off more carbon dioxide than the inactive muscle; still the elimination of carbon dioxide is increased considerably more than the absorption of oxygen. The respiratory quotient, $\frac{CO_2}{O}$, is found to be regularly raised during work; still this rise, which will be explained in detail in a following chapter on metabolism, can hardly be conditioned on the kind of processes going on in the muscle during activity with a sufficient supply of oxygen. In work a consumption of carbohydrates, glycogen, and sugar takes place. A consumption of sugar seems only to have been shown in muscle with blood circulation, while a consumption of glycogen also has been observed in removed muscle. The acid reaction of the muscle becomes greater with work. In regard to the extent of a re-formation of lactic acid opinion is divided. Respecting the behavior of fats in removed muscles nothing is known with certainty, though an increase in the consumption of fat in the organism has been observed in certain cases during activity. An increase in the nitrogenous extractive bodies of the creatin group seems also to occur. In regard to the proteid bodies the views are contradictory; but an increased elimination of nitrogen as a direct consequence of muscular exertion has thus far not been positively proved.

In close connection with the above-mentioned facts we have the question as to the origin of muscular activity so far as it has its origin in chemical processes. In the past the generally accepted opinion was that of LIEBIG, that the source of muscular action consisted of a metabolism of the proteid bodies; to-day another generally accepted view prevails. FICK and WISLICENUS[1] climbed the Faulhorn and calculated the amount of mechanical force expended in the attempt. With this they compared the mechanical equivalent transformed in the same time from the proteids, calculated from the nitrogen eliminated with the urine, and found that the work really performed was not by any means compensated by the consumption of proteid. It was therefore proved by this that proteids alone cannot be the source of muscular activity, and that this depends in great measure on the metabolism of non-nitrogenous substances. Many other observations have led to the same result, especially the experiments of VOIT, of PETTENKOFER and VOIT, and of other investigators, whose experiments show that while the elimination of nitrogen remains unchanged, the elimination of carbon dioxide during work is very considerably increased. It is also generally considered as positively proved that muscular work is produced, at least the greatest part, by the metabolism of non-nitrogenous substances. Nevertheless we are not warranted in the statement that muscular activity is produced entirely at the cost of the non-nitrogenous substances, and that the proteid bodies are without importance as a source of force.

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[1] Vierteljahrschr. d. Zürich. naturf. Gesellsch., Bd. 10. Cited from Centralbl. f. d. med. Wiss., 1866, S. 309.

The recent investigations of PFLÜGER[1] are of great interest in this connection. He fed a bulldog for more than 7 months with meat which alone did not contain sufficient fat and carbohydrates for the production of heart activity, and then let him work very hard for periods of 14, 35, and 41 days. The positive results obtained by these series of experiments was that "complete muscular activity may be effected to the greatest extent in the absence of fat and carbohydrates," and the ability of proteids to serve as a source of muscular energy cannot be denied.

Among the non-nitrogenous bodies we must accord to carbohydrates, glycogen, and sugar the first place as sources of force. That the fats are also to be considered as a source for force is very probable, and the researches of VOIT[2] on starving and working dogs give support to this theory. The view, as accepted by several investigators, that all three chief groups of organic food or muscle constituents may serve as source of force seems to be true. A few investigators are of the opinion, as formulated by BUNGE,[3] that the muscles first consume the supply of non-nitrogenous nutritive bodies, and that the proteids are only secondarily attacked. PFLÜGER is, on the contrary, of the opposite opinion. According to him no muscular work takes place without a decomposition of proteid, and the living cell substance prefers always the proteid and rejects the fat and sugar, contenting itself with these only when proteids are absent.

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[1] Pflüger's Arch., Bd. 50.

[2] Ueber den Einfluss des Kochsalzes, etc., l. c.

[3] Lehrbuch d. physiol. u. pathol. Chem., 1. Aufl., S. 345.

Zuntz,[1] in collaboration with Frentzel and Loeb, has made experiments in dogs from which he concludes, that at least in these experiments (part in starvation and part with such an abundant food that a deposition of nitrogen took place even after hard work) the animals preferred the non-nitrogenous bodies which were offered as food to defray the work done. Zuntz has also shown that the foods may supply work approximately in proportion as they consume oxygen and according to their heat of combustion.

Quantitative Composition of the Muscle. A large number of analyses have been made of the flesh of various animals for purely practical purposes, in order to determine the nutritive value of different varieties of meat; but we have no exact scientific analyses with sufficient regard to the quantity of different albuminous bodies and the remaining muscle-constituents, or these analyses are incomplete or of little value.

To give the reader some idea of the variable composition of muscle-substance we give the following summary, chiefly obtained from K. B. Hofmann's[2] book. The figures are parts per 1000.

	Muscles of Mammalia.	Muscles of Birds.	Muscles of Cold-blooded Animals.
Solids	217–255	227–282	200
Water	745–783	717–773	800
Organic bodies	208–245	217–263	180-190
Inorganic bodies	9–10	10–19	10-20
Myosin	35–106	29.8–111	29.7-87
Stroma substance (Danilewsky)	78–161	88.0–184	70.0-121
Alkali albuminate	29–30	—	—
Creatin	2	3.4	2.3
Xanthin bases	0.4–0.7	0.7–0.3	—
Inosinic acid (barium salt)	0.1	0.1–0.3	—
Protic acid	—	—	7.0
Taurin	0.7 (horse)	—	1.1
Inosit	0.03	—	—
Glycogen	4–5	—	3-5
Lactic acid	0.4–0.7	—	—
Phosphoric acid	3.4–4.8		
Potash	3.0–4.0		
Soda	0.4		
Lime	0.2		
Magnesia	0.4		
Sodium chloride	0.04–0.1		
Iron oxide	0.03–0.1		

In this table, which has little value because of the variation in the composition of the muscles, we have no results as to the estimates of fat. Owing to the variable quantity of fat in meat it is hardly possible to quote a positive average for this body. After most careful efforts to remove the fat from the muscles without chemical means, it has been found that a variable amount of intermuscular fat, which does not really belong to the muscular tissue, always remains. The smallest quantity of fat in the muscles from lean oxen is, according to GROUVEN, 6.1 p. m., and according to PETERSEN 7.6 p. m. This last observer also found regularly a smaller amount of fat, 7.6–8.6 p. m., in the fore quarter of oxen, and a greater amount, 30.1–34.6 p. m., in the hind quarter of the animal. A low amount of fat has also been found in the muscles of wild animals. B. KÖNIG and FARWICK found 10.7 p. m. fat in the muscles of the extremities of the hare, and 14.3 p. m. in the muscles of the partridge. The muscles of pigs and fattened animals are, when all the adherent fat is removed, very rich in fat, amounting to 40–90 p. m. The muscles of certain fishes also contain a large amount of fat. According to ALMÉN, the flesh of the salmon, mackerel, and eel contains respectively 100, 164, and 329 p. m. fat.[1]

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[1] Du Bois-Reymond's Arch., 1894.

[2] Lehrbuch d. Zoochem. Wien, 1876, S. 104.

The quantity of WATER in the muscle is liable to considerable variation. The amount of fat has a special influence on the quantity of water, and we find, as a rule, that the flesh which is deficient in water is correspondingly rich in fat. The quantity of water does not depend alone upon the amount of fat, but upon many other circumstances, among which we must mention the age of the animal. In young animals the organs in general, and therefore also the muscles, are poorer in solids and richer in water. In man the amount of water decreases until mature age, but increases again towards old age. Work and rest also influence the amount of water, for the active muscle contains more water than the inactive. The uninterruptedly active heart should therefore be the muscle richest in water. That the amount of water may vary independently of the amount of fat is strikingly shown by comparing the muscles of different species of animals. In cold-blooded animals the muscles generally have a greater amount of water, in birds a lower. The comparison of the flesh of cattle and fish shows very strikingly the different amounts of water (independent of the amount of fat) in the flesh of different animals. According to the analysis of ALMÉN,[1] the muscles of lean oxen contain 15 p. m. fat and 767 p. m. water; the flesh of the pike contains only 1.5 fat and 839 p. m. water.

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[1] In regard to the literature and complete statements on the composition of flesh of various animals, see König, Chemie der menschlichen Nahrungs- und Genussmittel, 3. Aufl.

For certain purposes, as, for example, in experiments on metabolism, it is important to know the elementary composition of flesh. In regard to the quantity of nitrogen we generally accept VOIT'S[2] figure, namely, 3.4%, as an average for fresh lean meat. According to NOWAK[3] and HUPPERT[4] this quantity may vary about 0.6%, and in more exact investigations it is therefore necessary to specially determine the nitrogen. According to SALKOWSKI,[5] of the total nitrogen of beef 77.4% was insoluble proteids, 10.08% soluble proteids, and 12.52% other soluble bodies. Complete elementary analyses of flesh have recently been made with great care by ARGUTINSKY.[6] The average for ox-flesh dried in vacuo and free from fat and with the glycogen deducted was as follows: C 49.6; H 6.9; N 15.3; O + S 23.0; and ash 5.2%. The relationship of the carbon to nitrogen, which ARGUTINSKY calls the "FLESH QUOTIENT," is on an average 3.24 : 1.

Non-striated Muscles.

The smooth muscles have a neutral or alkaline reaction (DU-BOIS-REYMOND)[7] when at rest. During activity they are acid, which is inferred from the observations of BERNSTEIN,[8] who found that the nearly continually contracting sphincter muscle of the *Anodonta* is acid during life. The smooth muscles may also, according to HEIDENHAIN[9] and KÜHNE,[10] pass into *rigor mortis* and thereby become acid. Because of this behavior it is believed that among the proteid bodies of the smooth muscles there is also a myosin-forming substance. A spontaneously coagulating plasma has not thus far been obtained, but it may be considered as the juice obtained by pressing the muscles of the Anodonta and which coagulates immediately at + 45° C. or within 24 hours at the ordinary temperature. Myosin has not been found in the smooth muscles. HEIDENHAIN and HELLWIG[1] have obtained from the smooth muscles of a dog an albuminous body which coagulates at +45° to 49° C. and which is analogous to musculin. The smooth muscles contain large amounts of alkali albuminates besides an albumin coagulating at + 75° C.

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[1] Nova Act. reg. Soc. Scient. Upsal., Vol. extr. ord., 1877; also Maly's Jahresber., Bd. 7, S. 307.

[2] Zeitschr. f. Biologie, Bd. 1.

[3] Wien. Sitzungsber., Bd. 64, Abth. 2.

[4] Zeitschr. f. Biologie, Bd. 7.

[5] Centralbl. f. d. med. Wissensch., 1894.

[6] Pflüger's Arch., Bd. 55.

[7] Cited by Nasse in Hermann's Handb., Bd. 1, S. 339.

[8] *Ibid.*

[9] *Ibid.*, S. 340.

[10] Lehrbuch. d. physiol. Chem., S. 331.

Hæmoglobin occurs in the smooth muscles of certain animals, but is absent in others. *Creatin* has been found by LEHMANN.[2] According to FRÉMY and VALENCIENNES,[3] the muscles of the Cephalopods contain *taurin* besides *creatinin* (*creatin?*). Of the non-nitrogenous substances, *glycogen* and *lactic acid* have been found without doubt. The mineral constituents show the remarkable fact that the sodium combinations exceed the potassium combinations.[4]

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[1] Nasse, l. c., S. 339.

[2] *Ibid.*

[3] Cited from Kühne's Lehrbuch, S. 333.

[4] *Ibid.*

CHAPTER XII.

BRAIN AND NERVES.

On account of the difficulty of making a mechanical separation and isolation of the different tissue-elements of the nervous central organ and the nerves, we must resort to a few microchemical reactions, chiefly to qualitative and quantitative investigations of the different parts of the brain, in order to study the different chemical composition of the cells and the nerve-tubes. The chemical investigation of this part is accompanied with the greatest difficulty; and although our knowledge of the chemical composition of the brain and nerves has been somewhat extended by the investigations of modern times, still we must admit that this chapter is as yet one of the most obscure and complicated in physiological chemistry.

Proteids of different kinds have been shown to be chemical constituents of the brain and nerves. A part of these are insoluble in water and dilute neutral-salt solutions, and part are soluble therein. Among the latter we find *albumin* and *globulin*. *Nucleoalbumin*, which is often considered as an alkali albuminate, also occurs. HALLIBURTON[1] found two globulins in the brain, one of which coagulates at 47–50° C. and the other at 70° C. He found in the gray matter a nucleoalbumin which coagulated at 55–60° C. and contained 0.5% phosphorus. It seems unquestionable that the albuminous bodies belong chiefly to the gray substance of the brain and to the axis-cylinders. The same remarks apply to *nuclein*, which v. JACKSCH[2] found in large quantities in the gray substance. *Neurokeratin* see page 49), which was first detected by KÜHNE, and which partly forms the *neuroglia*, and which as a double sheath envelops the outside of the nerve medulla under SCHWANN's sheath and the inner axis-cylinders, chiefly occurs in the white substance (KÜHNE and CHITTENDEN,[1] BAUMSTARK).[2]

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[1] On the Chemical Physiology of the Animal Cell. King's College, London, Physiological Laboratory. Collected Papers, No. 1, 1893.

[2] Pflüger's Arch., Bd. 13.

The phosphorized substance *protagon* must be considered as one of the chief constituents, perhaps the only constituent (BAUMSTARK), of the white substance. This last-mentioned substance, if we keep for the present to the most carefully studied protagon —because there are perhaps several different protagons—yields as decomposition products lecithin, fatty acids, and a nitrogenous substance, *cerebrin*; this last probably does not occur preformed in the brain, but is more likely a product of transformation. That *lecithin* also is pre-existent in the brain and nerves can hardly be doubted. The investigations thus far made have not shown decidedly whether it is more abundant in the gray or the white substance. *Fatty acids* and *neutral fats* may be prepared from the brain and nerves; but as these may be readily derived from a decomposition of lecithin and protagon, which exist in the fatty tissue between the nerve-tubes, it is difficult to decide what part the fatty acids and neutral fats play as constituents of the real nerve-substance. *Cholesterin* is also found in the brain and nerves, a part free and a part in chemical combination of which we know nothing about (BAUMSTARK). Cholesterin seems to occur in greater abundance in the white substance. Besides these substances the nerve tissue, especially the white substance, contains doubtless a number of other constituents not well known, and among which are several containing phosphorus. THUDICHUM asserted that he had isolated a number of phosphorized substances from the brain which he divided into three principal groups: *kepalines*, *myelines*, and *lecithines*. But thus far this assertion has not been confirmed by other investigators.

By allowing water to act on the contents of the medulla, round or oblong double-contoured drops or fibres, not unlike double-contoured nerves, are formed. This remarkable formation, which can also be seen in the medulla of the dead nerve, has been called "*myeline forms*," and they were formerly considered as produced from a special body, "myeline." Myeline forms may, however, be obtained from other bodies, such as impure protagon, lecithin, fat, and impure cholesterin, and they depend on a decomposition of the constituents of the medulla. According to GAD and HEYMANS[1] myeline is lecithin in a free condition or in loose chemical combination.

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[1] Zeitschr. f. Biologie, Bd. 26.

[2] Zeitschr. f. physiol. Chem., Bd. 9.

The *extractive bodies* seem to be almost the same as in the muscles. We find *creatin*, which may, however, be absent (BAUMSTARK), *xanthin bases, inosit, lactic acid* (also fermentation lactic acid), *uric acid, jecorin* (according to BALDI,[2] in the human brain), and *neuridin*, $C_5H_{14}N_2$, discovered by BRIEGER[3] and which is most interesting because of its appearance in the putrefaction of animal tissues or in cultures of the typhoid bacillus. Under pathological conditions *leucin* and *urea* have been found in the brain. Urea is also a physiological constituent of the brain of cartilaginous fishes.

Of the above-mentioned constituents of the nerve-substance protagon and its decomposition products, the cerebrins or cerebrosides, must be specially described.

Protagon. This body, which was discovered by LIEBREICH, is a nitrogenized and phosphorized substance whose elementary composition, according to GAMGEE and BLANKENHORN,[4] is C 66.39, H 10.69, N 2.39, and P 1.068 per cent. BAUMSTARK[5] and RUPPEL[6] obtained the same figures, while LIEBREICH[7] found an average of 2.80% N and 1.23% P. KOSSEL and FREYTAG,[8] who obtained still higher figures for the nitrogen, namely, 3.25%, and somewhat lower figures for the phosphorus, 0.97%, found some sulphur, an average of 0.51%, regularly in the protagon. RUPPEL also found some sulphur, but in such small quantity that he considered it as a contamination. On boiling with baryta-water protagon yields the decomposition products of lecithin, namely, fatty acids, glycerophosphoric acid, and cholin (neurin ?), and besides this also cerebrin. KOSSEL and FREYTAG found that protagon not only yielded cerebrin in its decomposition, but two and perhaps indeed three cerebrosides (see below), namely CEREBRIN, KERASIN (homocerebrin), and ENCEPHALIN. Because of this behavior, and also because of the varying elementary composition although the greatest care was taken in the preparation, FREYTAG considers it very probable that there are more than one protagon.

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[1] Du Bois-Reymond's Arch., 1890.

[2] *Ibid.*, 1887, Supplbd.

[3] Brieger, Ueber Ptomaine. Berlin, 1885 and 1886.

[4] Zeitschr. f. physiol. Chem., Bd. 3.

[5] *Ibid.*, Bd. 9.

[6] Zeitschr. f. Biologie, Bd. 31.

[7] Annal. d. Chem. u. Pharm., Bd. 134.

[8] Zeitschr. f. physiol. Chem., Bd. 17.

On boiling with dilute mineral acids, protagon yields among other substances a reducing carbohydrate. On oxidation with nitric acid protagon yields higher fatty acids.

Protagon appears, when dry, as a loose white powder. It dissolves in alcohol of 85 vols. per cent at + 45° C., but separates on cooling as a snow-white, flaky precipitate, consisting of balls or groups of fine crystalline needles. It decomposes on heating even below 100° C. It is hardly soluble in cold alcohol or ether, but dissolves on warming. It swells in little water, decomposes partly. With more water it swells to a gelatinous or pasty mass, which with much water yields an opalescent liquid. On fusing with saltpetre and soda, alkali phosphates are obtained.

Protagon is prepared in the following way: An ox-brain as fresh as possible, with the blood and membranes carefully removed, is ground fine and then extracted for several hours with alcohol of 85 vols. per cent at + 45° C., filtered at the same temperature, and the residue extracted with warm alcohol until the filtrate does not yield a precipitate at 0° C. The several alcoholic extracts are cooled to 0° C. and the precipitates united and completely extracted with cold ether, which dissolves the cholesterin and lecithin-like bodies. The residue is now strongly pressed between filter-paper and allowed to dry over sulphuric acid or phosphoric anhydride. It is now pulverized, digested with alcohol at + 45° C., filtered and slowly cooled to 0° C. The crystals which separate may be purified when necessary by recrystallization.

The same steps are taken when we wish to detect the presence of protagon.

On decomposing protagon or the protagons by the gentle action of alkalies we obtain as cleavage products, as above stated, one or more bodies, which THUDICHUM[1] has embraced under the name *cerebrosides*. The cerebrosides are nitrogenous substances free from phosphorus, which yield a reducing variety of sugar (galactose) on boiling with dilute mineral acids. On fusing with potash or by oxidation with nitric acid they yield higher fatty acids, palmitic or stearic acids. The cerebrosides isolated from the brain are cerebrin, kerasin, and encephalin. The bodies isolated by KOSSEL and FREYTAG from pus, pyosin and pyogenin, also belong to the cerebrosides.

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[1] Thudichum, Grundzüge der anatomischen und klinischen Chemie, Berlin, 1886.

Cerebrin. Under this name W. MÜLLER[1] first described a nitrogenous substance, free from phosphorus, which he obtained by extracting a brain-mass, which had been previously boiled with baryta-water, with boiling alcohol. Following a method essentially the same, but differing somewhat, GEOGHEGAN[2] prepared from the brain a cerebrin with the same properties as MÜLLER'S, but containing less nitrogen. According to PARCUS[3] the cerebrin isolated by GEOGHEGAN as well as by MÜLLER consists of a mixture of three bodies, "cerebrin," "homocerebrin," and "encephalin." KOSSEL and FREYTAG[4] isolated two cerebrosides from protagon which were identical with the cerebrin and homocerebrin of PARCUS. According to these investigators the two bodies phrenosin and kerasin as described by THUDICHUM seem to be identical with cerebrin and homocerebrin.

Cerebrin, according to PARCUS, has the following composition: C 69.08, H 11.47, N 2.13, O 17.23%, which corresponds with the analyses made by KOSSEL and FREYTAG. No formula has been given to this body. In the dry state it forms a pure white, odorless, and tasteless powder. On heating it melts, decomposes gradually, smells like burnt fat, and burns with a luminous flame. It is insoluble in water, dilute alkalies, or baryta-water. It is also insoluble in cold alcohol and in cold or hot ether. On the contrary, it is soluble in boiling alcohol and separates as a flaky precipitate on cooling, and this is found to consist of a mass of balls or grains on microscopical examination. Cerebrin forms an insoluble compound with baryta which decomposes by the action of carbon dioxide. Cerebrin dissolves in concentrated sulphuric acid, and on warming the solution it becomes blood-red. The variety of sugar split off on boiling with mineral acids—the so-called brain-sugar—is, according to THIERFELDER,[5] galactose.

Kerasin (according to THUDICHUM) or *homocerebrin* (according to PARCUS) has the following composition: C 70.06, H 11.60, N 2.23, and O 16.11%. *Encephalin* has the composition C 68.40, H 11.60, N 3.09, and O 16.91%. Both bodies remain in the mother liquor after the impure cerebrin has precipitated from the warm alcohol. These bodies have the tendency of separating as gelatinous masses. Kerasin is homologous to cerebrin, but dissolves more easily in warm alcohol and also in warm ether. It may be obtained as extremely fine needles. Encephalin is, according to PARCUS, a transformation product of cerebrin. In perfectly pure state it crystallizes in small lamellæ. It swells into a pasty mass in warm water. Like cerebrin and kerasin, it yields a reducing substance (probably galactose) on boiling with dilute acid.

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[1] Annal. d. Chem. u. Pharm., Bd. 105.

[2] Zeitschr. f. physiol. Chem., Bd. 3.

[3] Ueber einige neue Gehirnstoffe, Inaug.-Diss., Leipzig, 1881.

[4] L. c.

[5] Zeitschr. f. physiol. Chem., Bd. 14.

The cerebrins are generally prepared according to MÜLLER'S method. The brain is first stirred with baryta-water until it appears like thin milk and then it is boiled. The insoluble parts are removed, pressed, and repeatedly boiled with alcohol, which is filtered while boiling hot. The impure cerebrin which separates on cooling is freed from cholesterin and fat by means of ether, and then purified by repeated solution in warm alcohol. According to PARCUS this repeated solution in alcohol is continued until no gelatinous separation of homocerebrin or encephalin takes place.

According to GEOGHEGAN'S method the brain is first extracted with cold alcohol and ether and then boiled with alcohol. The precipitate which separates on the cooling of the alcoholic filtrate is treated with ether and then boiled with baryta-water. The insoluble residue is purified by repeated solution in boiling alcohol.

The cerebrin may also be obtained from other organs by employing the above methods. The quantitative estimation, when such is desired, may be performed in the same way.

KOSSEL and FREYTAG prepare cerebrin from protagon by saponifying it in a solution in methyl alcohol with a hot solution of caustic baryta in methyl alcohol. The precipitate is filtered off and decomposed in water by carbon dioxide, and the cerebrin or cerebroside extracted from the insoluble residue by hot alcohol.

Neuridin, $C_5H_{14}N_2$, is a non-poisonous diamin discovered by BRIEGER, and which was obtained by him in the putrefaction of meat and gelatine, and from cultures of the typhoid bacillus. It also occurs under physiological conditions in the brain, and as traces in the yolk of the egg.

Neuridin dissolves in water, and yields on boiling with alkalies a mixture of dimethylamin and trimethylamin. It dissolves with difficulty in amyl-alcohol. It is insoluble in ether or absolute alcohol. In the free state neuridin has a peculiar odor, suggesting semen. With hydrochloric acid it gives a combination crystallizing in long needles. With platinic chloride or gold chloride it gives crystallizable double combinations which are valuable in its preparation and detection.

The so-called CORPUSCULA AMYLACEA, which occur on the upper surface of the brain and in the pituitary gland, are colored more or less pure violet by iodine and more blue by sulphuric acid and iodine. They consist, perhaps, of the same substance as certain prostatic calculi, but they have not been closely investigated.

Quantitative Composition of the Brain. The quantity of water is greater in the gray than in the white substance, and greater in new-born or young individuals than in grown ones. The brain of the fœtus contains 879–926 p. m. water. According to the observations of Weisbach[1] the amount of water in the several parts of the brain (and in the medulla) varies at different ages. The following figures are in 1000 parts—*A* for men and *B* for women:

	20–30 Years.		30–50 Years.		50–70 Years.		70 94 Years.	
	A.	*B.*	*A.*	*B.*	*A.*	*B.*	*A.*	*B.*
White substance of the brain	695.6	682.9	683.1	703.1	701.9	689.6	726.1	722 0
Gray ditto	833.6	826.2	836.1	830.6	838.0	838.4	847.8	839.5
Gyri	784.7	792.0	795 9	772.9	796.1	796.9	802.3	801.7
Cerebellum	788.3	794.9	778.7	789.0	787.9	784.5	803.4	797.9
Pons varoli	734.6	740.3	725.5	722.0	720.1	714.0	727.4	724.4
Medulla oblongata	744.3	740.7	732.5	729.8	722.4	730 6	736.2	733.7

Quantitative analyses of the brain have also been made by Petrowsky[2] on an ox-brain and by Baumstark[3] on the brain of a horse. In the analysis of Petrowsky the protagon has not been considered, and all organic, phosphorized substances were calculated as lecithin. On these grounds these analyses are not of much value from a certain standpoint. In Baumstark's analyses the gray and the white substance could not be sufficiently separated, and these analyses, on this account, show partly an excess of white and partly an excess of gray substance; nearly one half of the organic bodies, chiefly consisting of bodies soluble in ether, could not be exactly analyzed. Neither of these analyses gives sufficient explanation of the quantitative composition of the brain.

The analyses made up to the present time give, as above stated, an unequal division of the organic constituents in the gray and white substance. In the analyses of Petrowsky the quantity of proteids and gelatin-forming substances in the gray matter was somewhat more than one half, and in the white about one quarter, of the solid organic substances. The quantity of cholesterin in the white was about one half, and in the gray substance about one fifth, of the solid bodies. A greater quantity of soluble salts and extractive bodies was found in the gray substance than in the white (Baumstark). The following analyses of Baumstark give the most important known constituents of the brain calculated in 1000 parts of the fresh, moist brain. *A* represents chiefly the white, and *B* chiefly the gray, substance.

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[1] Cited from K. B. Hofmann's Lehrb. d. Zoochemie (Wien, 1876), S. 121.

[2] Pflüger's Arch., Bd. 7.

[3] Zeitschr. f. physiol. Chem., Bd. 9.

	A.	*B.*
Water	695.35	769.97
Solids	304.65	230.03
Protagon	25.11	10.80
Insoluble proteid and connective tissue	50.02	60.79
Cholesterin, free	18.19	6.30
" combined	26.96	17.51
Nuclein	2.94	1.99
Neurokeratin	18.93	10.43
Mineral bodies	5.23	5.62

The remainder of the solids probably consists chiefly of lecithin and other phosphorized bodies. Of the total amount of phosphorus 15–20 p. m. belongs to the nuclein, 50–60 p. m. to the protagon, 150–160 p. m. to the ash, and 770 p. m. to the lecithin and the other phosphorized organic substances.

The quantity of neurokeratin in the nerves and in the different parts of the brain has been carefully determined by KÜHNE and CHITTENDEN.[1] They found 3.16 p. m. in the plexus brachialis, 3.12 p. m. in the edge of the cerebellum, 22.434 p. m. in the white substance of the cerebrum, 25.72–29.02 p. m. in the white substance of the corpus callosum, and 3.27 p. m. in the gray substance of the edge of the cerebrum (when free as possible from white substance). The white is very considerably richer in neurokeratin than the peripheric nerves or the gray substance. According to GRIFFITHS[2] neurochitin replaces neurokeratin in insects and crustacea, the quantity of the first being 10.6–12 p. m.

The quantity of mineral constituents in the brain amounts to 2.95–7.08 p. m. according to GEOGHEGAN.[3] He found in 1000 parts of the fresh, moist brain 0.43–1.32 Cl, 0.956–2.016 PO_4, 0.244–0.796 CO_2, 0.102–0.220 SO_4, 0.01–0.098 $Fe_2(PO_4)_2$, 0.005–0.022 Ca, 0.016–0.072 Mg, 0.58–1.778 K, 0.450–1.114 Na. The gray substance yields an alkaline ash, the white an acid ash.

Appendix.

The Tissue and Fluids of the Eye.

The retina contains in all 865–899.9 p. m. water, 57.1–84.5 p. m. proteid bodies—myosin, albumin, and mucin (?), 9.5–28.9 p. m. lecithin, and 8.2–11.2 p. m. salts (HOPPE-SEYLER and

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[1] Zeitschr. f. Biologie, Bd. 26.

[2] Compt. rend., Tome 115.

[3] Zeitschr. f. physiol. Chem., Bd. 3.

CAHN[1]). The mineral bodies consist of 422 p. m. Na_2HPO_4 and 352 p. m. NaCl.

Those bodies which form the different segments of the rods and cones have not been closely studied, and the greatest interest is therefore connected with the coloring matters of the retina.

Visual purple, also called *rhodopsin*, *erythropsin*, or VISUAL RED, is the pigment of the rods. BOLL[2] observed in 1876 that the layer of rods in the retina during life had a purplish-red color which was bleached by the action of light. KÜHNE[3] showed later that this red color might remain for a long time after the death of the animal if the eye was protected from daylight or investigated by a sodium light. Under these conditions it was also possible to isolate and closely study this substance.

Visual red (BOLL) or visual purple (KÜHNE) has become known mainly by the investigations of KÜHNE. The pigment occurs chiefly in the rods and only in their outer parts. In animals whose retina has no rods the visual purple is absent, and is also necessarily absent in the macula lutea. In a variety of bat (*rhinolophus hipposideros*), in hens, pigeons, and new-born rabbits, no visual purple has been found in the rods.

A solution of visual purple in water which contains 2–5% crystallized bile, which is the best solvent for it, is purple-red in color, quite clear, and not fluorescent. On evaporating this solution *in vacuo* we obtain a residue similar to ammonium carminate which contains violet or black grains. If the above solution is dialyzed with water, the bile diffuses and the visual purple separates as a violet mass. Under all circumstances, even when still in the retina, the visual purple is quickly bleached by direct sunlight, and with diffused light with a rapidity corresponding to the intensity of the light. It passes from red and orange to yellow. Red light bleaches the visual purple slowly; the ultra-red light does not bleach it at all. A solution of visual purple shows no special absorption-bands, but only a general absorption which extends from the red side, beginning at *D*, to the line *G*. The strongest absorption is found at *E*.

Visual purple when heated to 52°–53° C. is destroyed after several hours, and almost instantly when heated to + 76° C. It is also destroyed by alkalies, acids, alcohol, ether, and chloroform. On the contrary, it resists the action of ammonia or alum solution.

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[1] Zeitschr f. Physiol. Chem., Bd. 5.

[2] Monatsschr. d. Berl. Akad., 12 Nov. 1876.

[3] The investigations of Kühne and his pupils Ewald and Ayres on the visual purple will be found in Untersuchungen aus dem physiol. Institut der Universität Heidelberg, Bdd. 1 und 2.

As the visual purple is easily destroyed by light, it must therefore also be regenerated during life. KÜHNE has also found that the retina of the eye of the frog becomes bleached when exposed for a long time to strong sunlight, and that its color gradually returns when the animal is placed in the dark. This regeneration of the visual purple is a function of the living cells in the layer of the pigment-epithelium of the retina. This may be inferred from the fact that a detached piece of the retina which has been bleached by light may have its visual purple restored if the detached piece of the retina be carefully laid on the chorioïdea having layers of the pigment-epithelium attached. The regeneration has, it seems, nothing to do with the dark pigment, the melanin or fuscin, in the epithelium-cells. A partial regeneration seems, according to KÜHNE, to be possible in the completely removed retina. On account of this property of the visual purple of being bleached by light during life we may, as KÜHNE has shown, under special conditions and by observing special precautions, obtain after death by the action of intense light or more continuous light the picture of bright objects, such as windows and the like—so-called optograms.

The physiological importance of visual purple is unknown. It follows that the visual purple is not essential to sight, since it is absent in certain animals and also in the cones.

Visual purple must always be prepared exclusively in a sodium light. It is extracted from the net membrane by means of a watery solution of crystallized bile. The filtered solution is evaporated *in vacuo* or dialyzed until the visual purple is separated.

To prepare a visual-purple solution, perfectly free from hæmoglobin, KÜHNE[1] suggests to precipitate it from it solution in bile by $Mg\ SO_4$ in substance, or to treat the retina, which has been previously hardened by alum and then lixiviated with water and 10% NaCl solution, with bile.

The pigments of the cones. In the inner segments of the cones of birds, reptiles, and fishes a small fat-globule of varying color is found. KÜHNE[2] has isolated from this fat a green, a yellow, and a red pigment called respectively *chlorophan*, *xanthophan*, and *rhodophan*.

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[1] Zeitschr. f. Biologie, Bd. 32.

[2] Kühne, Die nichtbeständigen Farben der Netzhaut. Untersuch. aus dem physiol. Institut Heidelberg, Bd. 1, S. 341.

The dark pigment of the epithelium-cells of the net membrane, which was formerly called *melanin*, but since named *fuscin* by **Kühne** and **May**,[1] dissolves in concentrated caustic alkalies or concentrated sulphuric acid on warming, but, like melanins in general (see Chapter XVI), has been little studied. The pigment occurring in the pigment-cells of the chorioïdea seems to be identical with the fuscin of the retina.

The **vitreous humor** is often considered as a variety of gelatinous tissue. The membrane consists, according to C. Mörner,[2] of a gelatine-forming substance. The fluid contains a little proteid and a mucoid, *hyalomucoid*, which was first shown by Mörner, and which is not precipitated by acetic acid. This contains 12.27% N and 1.19% S. Among the extractives we find a little *urea* according to Picard[3] 5 p. m., according to Rählmann[4] 0.64 p. m. Pautz[5] found besides some urea also paralactic acid, and, in confirmation of the statements of Chabbas, Jesner, and Kuhn, also glucose in the vitreous humor of oxen. The reaction of the vitreous humor is alkaline, and the quantity of solids amounts to about 11 p. m. The quantity of mineral bodies is about 9 p. m., and the albuminous bodies 0.7 p. m. In regard to the aqueous humor see page 195.

The **crystalline lens.** That substance which forms the capsule of the lens; has been recently investigated by C. Mörner. It belongs, according to him, to a special group of protein, which are called *membranins*. The membranin bodies are insoluble at the ordinary temperature in water, salt solutions, dilute acids and alkalies, and, like the mucins, yield a reducing substance on boiling with dilute mineral acids. They contain sulphur, which blackens lead. The membranins are colored a very beautiful red by Millon's reagent, but give no characteristic reaction with concentrated hydrochloric acid or Adamkiewicz's reagent. They are dissolved with great difficulty by pepsin-hydrochloric acid or trypsin solution. They are dissolved by dilute acids and alkalies in the warmth. Membranin of the capsule of the lens contains 14.10% N and 0.83% S, and is a little less soluble than that from Descemets membrane.

The chief mass of the solids of the crystalline lens consists of proteids, whose nature has been investigated by C. MÖRNER.[1] Some of these proteids are insoluble in dilute salt solution, and others soluble therein.

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[1] Kühne, *ibid.*, Bd. 2, S. 324.

[2] Zeitschr. f. physiol. Chem., Bd. 18.

[3] Gamgee's Physiol. Chem., p. 454.

[4] Maly's Jahresber., Bd. 6, S. 219.

[5] Zeitschr. f. Biologie, Bd. 31.

The Insoluble Proteid. The lens-fibres consist of a proteid substance which is insoluble in water and salt solution to which MÖRNER has given the name ALBUMOID. It dissolves readily in very dilute acids or alkalies. Its solution in caustic potash of 0.1% is very similar to an alkali-albuminate solution, but coagulates at about 50° C. on nearly complete neutralization and addition of 8% NaCl. Albumoid has the following composition: C 53.12, H 6.8, N 16.62, and S 0.79%. The lens-fibres themselves contain 16.61% N and 0.77% S. The inner parts of the lens are considerably richer in albumoid than the outer. The quantity of albumoid in the entire lens amounts on an average to about 48% of the total weight of proteids of the lens.

The Soluble Proteid consists, exclusive of a very small quantity of ALBUMIN, of two globulins, α- and β-CRYSTALLIN. These two globulins differ from each other in this manner: α-crystallin contains 16.68% N and 0.56% S; β-crystallin, on the contrary, 17.04% N and 1.27% S. The first coagulates at about 72° C. and the other at 63° C. Besides this, β-crystallin is precipitated from salt-free solution with greater difficulty by acetic acid or carbon dioxide. These globulins are not precipitated by an excess of NaCl at either the ordinary temperature or 30° C. Magnesium or sodium sulphate in substance precipitates both globulins, on the contrary, at 30° C. These two globulins are not equally divided in the mass of the lens. The quantity of α-crystallin diminishes in the lens from without inwards, β-crystallin, on the contrary, from within outwards.

A BÉCHAMP[2] distinguishes the two following albuminous bodies in the watery extract of the crystalline lens: *phacozymase*, which coagulates at $+ 55°$ C., and contains a diastatic enzyme, and has a specific rotatory power of $(\alpha)j = - 41°$, and the *crystalbumin*, with a specific rotatory power of $(\alpha)j = - 80°.3$. From the residue of the lens, which was insoluble in water, BÉCHAMP extracted, by means of hydrochloric acid, an albuminous body having a specific rotatory power of $(\alpha)j = - 80°.2$ which he called *crystalfibrin*.

The lens does not seem to contain any proteid bodies which coagulate spontaneously like fibrinogen. That cloudiness which appears after death depends, according to KÜNHE,[3] upon the unequal changing of the concentration of the contents of the lens-tubes. This change is produced by the altered ratio of diffusion. A cloudiness of the lens may also be produced in life by a rapid removal of water, as, for example, when a frog is plunged into a salt or sugar solution (KUNDE[1]). The appearance of cloudiness in diabetes has been attributed by some to the removal of water. The views on this subject are, however, contradictory.

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[1] Zeitschr. f. physiol. Chem., Bd. 18. This contains also the pertinent literature.

[2] Compt. rend., Bd. 90.

[3] Lehrbuch d. physiol. Chem., S. 405.

The average results of four analyses made by LAPTSCHINSKY[2] of the lens of oxen are here given, calculated in parts per 1000:

Proteids	349.3
Lecithin	2.3
Cholesterin	2.2
Fat	2.9
Soluble salts	5.3
Insoluble salts	2.3

In cataract the amount of proteid is diminished and the amount of cholesterin increased.

The quantity of the different proteids in the fresh moist lens of oxen is as follows, according to MÖRNER[3]:

Albumoid (lens-fibres)	170 p. m.
β-crystallin	110 "
α-crystallin	68 "
Albumin	2 "

The **corneal tissue** has been previously treated of (page 348). The **sclerotic** has not been closely investigated, and the **choroid coat** is chiefly of interest because of the coloring matter, melanin, it contains (see Chap. XVI).

TEARS consist of a water-clear, alkaline fluid of a saltish taste. According to the analyses of LERCH[4] they contain 982 p. m. water, 18 p. m. solids, with 5 p. m. albumin and 13 p. m. NaCl.

The Fluids of the Inner Ear.

The **perilymph** and **endolymph** are alkaline fluids which, besides salts, contain—in the same amounts as in transudations—traces of *albumin*, and in certain animals (codfish) also *mucin*. The quantity of mucin is greater in the perilymph than in the endolymph.

Otoliths contain 745–795 p. m. inorganic substance, which consists chiefly of crystallized calcium carbonate. The organic substance is very like mucin.

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[1] Cited from Kühne, l. c.

[2] Pflüger's Arch., Bd. 13.

[3] L. c.

[4] Cited from Gorup-Besanez, Lehrb. d. physiol Chem., 4. Aufl., S. 401.

CHAPTER XIII.

ORGANS OF GENERATION.

(a) Male Generative Secretions.

THE **testis** have been little investigated chemically. We find in the testis of animals proteid bodies of different kinds, *seralbumin, alkali albuminate* (?), and an albuminous body related to ROVIDAS' *hyaline substance*, also *leucin, tyrosin, creatin, xanthin bases, cholesterin, lecithin, inosit*, and *fat*. In regard to the occurrence of glycogen the statements are somewhat contradictory. DARESTE[1] found in the testis of birds starch-like granules, which were colored blue with difficulty by iodine.

The **semen** as ejected is a white or whitish-yellow, viscous, sticky fluid of a milky appearance, with whitish, non-transparent lumps. The milky appearance is due to spermatozoa. Semen is heavier than water, contains albumin, has a neutral or faintly alkaline reaction and a peculiar specific odor. Soon after ejection semen becomes gelatinous, as if it were coagulated, but afterwards becomes more fluid. When diluted with water white flakes or shreds separate (HENLE'S *fibrin*). According to the analyses of VAUQUELIN[2] human semen contains 900 p. m. water and 100 p. m. solids, with 60 p. m. organic and 40 p. m. inorganic substance, of which 30 p. m. is calcium phosphate. Among the albuminous bodies POSNER[3] claims that *propeptone* occurs even in the absence of the spermatozoa.

The semen in the vas deferens differs chiefly from the ejected semen in that it is without the peculiar odor. This last depends on the admixture with the secretion of the prostate. This secretion, according to IVERSEN,[1] has a milky appearance and ordinarily an alkaline reaction, very rarely a neutral one, contains small amounts of proteids and mineral bodies, especially NaCl. Besides these it contains a crystalline combination of phosphoric acid with a base, C_2H_5N. This combination has been called BÖTTCHER'S *spermine crystals*, and it is claimed that the specific odor of the semen is due to a partial decomposition of these crystals.

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[1] Compt. rend., Tome 74.

[2] Cited from Lehmann's Lehrb. d. physiol. Chem. (Leipzig, 1853), Bd. 2, S. 303.

[3] Berlin. klin. Wochenschr., 1888, No. 21, and Centralbl. f. d. med. Wiss., 1890, S. 497.

The crystals which appear on slowly evaporating the semen, and which are also observed in anatomical preparations kept in alcohol and in desiccated egg-albumin, are identical, according to SCHREINER, with CHARCOT'S crystals found in the blood, and in the lymphatic glands in leucæmia. They are, according to SCHREINER,[2] a combination of phosphoric acid with a base, *spermin*, C_2H_5N, which he discovered.

Spermin. The views in regard to the nature of this base are not unanimous. According to the investigations of LADENBURG and ABEL[3] it is not improbable that spermin is identical with ethylenimin, but this identity is disputed by MAJERT and A. SCHMIDT,[4] and also by POEHL.[5] The compound of spermin with phosphoric acid—Böttcher's spermine crystals—is insoluble in alcohol, ether, and chloroform, soluble with difficulty in cold water but more readily in hot water, and easily soluble in dilute acids or alkalies, also alkali carbonates and ammonia. The base is precipitated by tannic acid, mercuric chloride, gold chloride, platinic chloride, potassium-bismuthic iodide, and phosphotungstic acid. Spermin has a tonic action, and according to POEHL[6] it has a marked action on the oxidation processes of the animal body.

The **spermatozoa** show a great resistance to chemical reagents in general. They do not dissolve completely in concentrated sulphuric acid, nitric acid, acetic acid, nor in boiling-hot soda solutions. They are soluble in a boiling-hot caustic-potash solution. They resist putrefaction, and after drying they may be obtained again in their original form by moistening them with a 1% common-salt solution. By careful heating and burning to an ash the shape of the spermatozoa may be seen in the ash. The quantity of ash is about 50 p. m. and consists mainly ($\frac{3}{4}$) of potassium phosphate.

The spermatozoa show well-known movements, but the cause of this is not known. This movement may continue for a very long time, as under some conditions it may be observed for several days in the body after death, and in the secretion of the uterus longer than a week. Acid liquids stop these movements immediately; they are also destroyed by strong alkalies, especially ammoniacal liquids, also by distilled water, alcohol, ether, etc. The movements continue for a longer time in faintly alkaline liquids, especially in alkaline animal secretions, and also in properly diluted neutral salt-solutions.

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[1] Nord. med. Ark., Bd. 6; also Maly's Jahresber., Bd. 4, S. 358.

[2] Annal. d. Chem. u. Pharm., Bd. 194.

[3] Ber. d. deutsch. chem. Gesellsch., Bd. 21.

[4] *Ibid.*, Bd. 24.

[5] Compt. rend., Tome 115.

[6] Berlin. klin. Wochenschr., 1893, No. 36.

According to the investigations of MIESCHER,[1] there are *lecithin* and *nuclein*, but no cerebrin, in the SPERMATOZOA of BULLS. The head of the spermatozoa contains nuclein, which forms probably the outer part of the head; *albumin*, which forms the contents of the head; and lastly a substance rich in sulphur which has not been studied. The tail dissolves in gastric juice after continuous digestion, and seems to consist of proteids or allied bodies which show a variable resistance towards pepsin-hydrochloric acid.

The SPERMATOZOA of the RHINE SALMON show, according to MIESCHER, a great resistance. With caustic-potash and soda solutions they give a cloudy, gelatinous mass which is precipitated as shreds by acids; but these shreds do not dissolve in an excess of the acid. They are strongly attacked by a 10–15% solution of NaCl or $NaNO_3$, and the semen is converted by such a solution into a stiff gelatin. The head is attacked, but not the tail or the middle part. This last-mentioned part, like the tail, contains albumin, which is dissolved by hydrochloric acid of 1 p. m., but not in NaCl. MIESCHER also found *lecithin, fat, cholesterin, guanin*, and *sarkin* in relatively large amounts in the salmon-semen. The organic constituent occurring in the largest amount in the salmon-semen is, according to MEISCHER, a combination of *nuclein* with the base *protamin*, which is soluble in water but insoluble in alcohol or ether. According to KOSSEL, the nuclein of the spermatozoa is nucleic acid (see Chapter V), and a combination of nucleic acid and protamin is supposed to exist therein.

Protamin. This base is, like its salts, hardly possible to obtain in perfectly characteristic crystals. The platinum double salt has the following composition, according to PICCARD[2]: $PtCl_4 + 2(HCl.C_8H_{16}N_{4\frac{1}{2}}O_2)$. The compounds with hydrochloric or nitric acid dissolve readily in water and with difficulty in alcohol. They are insoluble in ether. The base is precipitated by silver nitrate, potassium-mercuric iodide, potassium ferricyanide, and phosphomolybdic acid.

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[1] Verh. d. naturf. Gesellsch. in Basel, Bd. 6; also Maly's Jahresber., Bd. 4, S. 337.

[2] Maly's Jahresber., Bd. 4, S. 355.

According to Miescher, the spermatozoa of salmon contain 487 p. m. nuclein, 268 p. m. protamin, 103 p. m. proteid bodies, 75 p. m. lecithin, 22 p. m. cholesterin, and 45 p. m. fat. Piccard found 60–80 p. m. guanin and sarkin in ripe semen. Kossel and Schindler[1] found no guanin, but *xanthin* and large amounts of adenin and hypoxanthin, in the semen of the carp.

Inoko,[2] who investigated the semen of bulls, boars, and salmon, found the four ordinary nuclein bases in all. The xanthin bases occurred habitually in greater quantity than the sarkin bases, and the relationship between the two was very variable.

Spermatin is a name which has been given to a constituent similar to alkali albuminate, but it has not been closely studied.

Prostatic concrements are of two kinds. One is very small, generally oval in shape with concentric layers. In young but not in older persons they are colored blue by iodine (Iversen).[3] The other kind is larger, sometimes the size of the head of a pin, and consisting chiefly of calcium phosphate (about 700 p. m.) with only a very small amount, about 160 p. m., organic substance.

(b) Female Generative Organs.

The stroma of the **ovaries** are of little interest from a physiologico-chemical standpoint, and the most important constituent of the ovaries, the Graaffian *follicles* with the *ovum*, have thus far not been the subject of a careful chemical investigation. The fluid in the follicles (of the cow) do not contain, as has been stated, the peculiar bodies, paralbumin or metalbumin, which are found in certain pathological ovarial fluids, but seems to be a serous liquid. The *corpora lutea* are colored yellow by an amorphous pigment called *lutein*. Besides this, another coloring-matter sometimes occurs which is not soluble in alkali; it is crystalline, but not identical with bilirubin or hæmatoidin; but it may be identified as a lutein by its spectroscopic behavior (Piccolo and Lieben, Kühne and Ewald).[4]

The cysts often occurring in the ovaries are of special pathological interest, and these may have essentially different contents, depending upon their variety and origin.

The **serous cysts** (Hydrops folliculorum Graafii), which are formed by a dilation of the Graafian follicles, contain a serous liquid which has a specific gravity of 1.005–1.022. A specific gravity of 1.020 is less frequent. Generally the specific gravity is lower, 1.005–1.014, with 10–40 p. m. solids. As far as is known, the contents of these cysts do not essentially differ from other serous liquids.

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[1] Zeitschr. f. physiol. Chem., Bd.

[2] *Ibid.*, Bd. 18.

[3] L. c.

[4] See Chapter VI, p. 145.

The **proliferous cysts** (MYXOID CYSTS, COLLOID CYSTS), which are developed from PFLÜGER's epithelium-tubes, may have a contents of a very variable composition.

We sometimes find in small cysts a semi-solid, transparent, or somewhat cloudy or opalescent mass which appears like solidified glue or quivering jelly, and which has been called *colloid* because of its physical properties. In other cases the cysts contain a thick, tough mass which can be drawn out into long threads, and as this mass in the different cysts is more or less diluted with serous liquids their contents may have a variable consistency. In other cases the small cysts may also contain a thin, watery fluid. The color of the contents is also variable. In certain cases they are bluish white, opalescent, and in others yellow, yellowish brown, or yellowish with a shade of green. They are often colored more or less chocolate-brown or red-brown, due to the decomposed blood-coloring matters. The reaction is alkaline or nearly neutral. The specific gravity, which may vary considerably, is generally 1.015–1.030, but may in few cases be 1.005–1.010 or 1.050–1.055. The amount of solids is very variable. In rare cases they amount to only 10–20 p. m.; ordinarily they vary between 50–70–100 p. m. In a few cases 150–200 p. m. solids have been found.

As form-elements we find red and white *blood-corpuscles*, *granular cells*, partly fat-degenerated epithelium and partly large so-called GLUGE's corpuscles, *fine granular masses*, *epithelium-cells*, *cholesterin crystals*, and *colloid corpuscles*—large, circular, highly refractive formations.

Though the contents of the proliferous cyst may have a variable composition, still it may be characterized in typical cases by its slimy or ropy consistency; by its grayish-yellow, chocolate-brown, sometimes whitish-gray color; and by its relatively high specific gravity, 1.015–1.025. Such a liquid does not ordinarily show a spontaneous fibrin-coagulation.

We consider *colloid*, *metalbumin*, and *paralbumin* as characteristic constituents of these cysts.

Colloid. This name does not designate any particular chemical substance, but is given to the contents of tumors with certain physical properties similar to gelatin jelly. Colloid is found as a diseased product in several organs.

Colloid is a gelatinous mass, insoluble in water and acetic acid; it is dissolved by alkalies and gives a liquid which is not precipitated by acetic acid or by acetic acid and potassium ferrocyanide. According to Pfannenstiel[1] such a colloid is designated β-pseudomucin.

Sometimes a colloid is found which, when treated with a very dilute alkali, gives a solution similar to a mucin solution. On boiling with acids colloid gives a reducing substance. It is related to mucin, and it is considered by certain investigators as a transformed mucin. A colloid found by Wurtz[2] in the lungs contains C 48.09, H 7.47, N 7.00, and O 37.44 %. Colloids of different origin seem to have an unequal composition.

Metalbumin. This name Scherer[3] gave to a protein substance found by him in an ovarial fluid. The metalbumin was considered by Scherer to be an albuminous body, but it belongs to the mucin group, and it is for this reason called *pseudomucin* by the author.[4]

Pseudomucin. This body, which, like mucin, gives a reducing substance when boiled with acids, is a mucoid of the following composition: C 49.75, H 6.98, N 10.28, S 1.25, O 31.74 % (Author). With water pseudomucin gives a slimy, ropy solution, and it is this substance which gives the fluid contents of the ovarial cysts their typical ropy property. Its solutions do not coagulate on boiling, but only become milky-opalescent. Unlike mucin solutions, pseudomucin solutions are not precipitated by acetic acid. With alcohol they give a coarse flocculent or thready precipitate which is soluble even after having been kept under water or alcohol for a long time. Mitjukoff[5] has isolated and investigated a colloid from an ovarial cyst. It had the following composition: C 51.76, H 7.76, N. 10.7, S 1.09, and O 28.69 %, and differed from mucin and pseudomucin by reducing FEHLING's solution before boiling with acid. He calls it *paramucin.*

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[1] Arch. f. Gynäk., Bd. 38.

[2] See Lebert, Beitr. zur Kenntniss des Gallertkrebses, Virchow's Arch., Bd. 4.

[3] Verh. d. physik.-med. Gesellsch. in Würzburg, Bd. 2, and Sitzungsber. der physik.-med. Gesellsch. in Würzburg für 1864–1865; Würzburg med. Zeitschr., Bd. 7.

[4] Zeitschr. f. physiol. Chem., Bd. 6.

[5] Ueber das paramucin. Inaug.-Diss., Berlin, 1895.

Paralbumin is another substance discovered by SCHERER,[1] and which occurs in ovarial liquids and also in ascites fluids with the simultaneous presence of ovarial cysts and rupture of the same. It is therefore only a mixture of pseudomucin with variable amounts of proteid, and the reactions of paralbumin are correspondingly variable.

The detection of metalbumin and paralbumin is naturally connected with the detection of pseudomucin. A typical ovarial fluid containing pseudomucin is, as a rule, sufficiently characterized by its physical properties, and a special chemical investigation is only necessary in cases where a serous fluid contains very small amounts of pseudomucin. We proceed in the following way: The proteid is removed by heating to boiling with the addition of acetic acid; the filtrate is strongly concentrated and precipitated by alcohol. The precipitate is carefully washed with alcohol, and then dissolved in water. A part of this solution is digested with saliva at the temperature of the body and then tested for glucose (derived from glycogen or dextrin). If glycogen is present, it will be converted into glucose by the saliva; precipitate again with alcohol and then proceed as in the absence of glycogen. In this last-mentioned case, first add acetic acid to the solution of the alcohol precipitate in water so as to precipitate any existing mucin. The precipitate produced is filtered, the filtrate treated with 2% HCl, and warmed on the water-bath until the liquid is deep brown in color. In the presence of pseudomucin this solution gives TROMMER's test.

The other protein bodies which have been found in cystic fluids are *serglobulin* and *seralbumin*, *peptone* (?), *mucin*, and *mucin-peptone* (?). Fibrin only occurs in exceptional cases. The quantity of mineral bodies on an average amounts to about 10 p. m. The amount of extractive bodies (*cholesterin* and *urea*) and *fat* is ordinarily 2–4 p. m. The remaining solids, which constitute the chief mass, are albuminous bodies and pseudomucin.

The **intraligamentary, papillary cysts** contain a yellow, yellowish-green, or brownish-green liquid which contains either no pseudo-mucin or very little. The specific gravity is generally rather high, 1.032–1.036, with 90–100 p. m. solids. The principal constituents are the albuminous bodies of blood-serum.

The rare **tubo-ovarial cysts** contain as a rule a watery, serous fluid containing no pseudomucin.

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[1] L. c.

The **parovarial cysts** or the CYSTS of the LIGAMENTA LATA may attain a considerable size. In general, and when quite typical, the contents are watery, mostly very pale yellow-colored, water-clear or only slightly opalescent liquids. The specific gravity is low, 1.002-1.009; and the solids only amount to 10–20 p. m. Pseudomucin does not occur as a typical constituent; proteid is sometimes absent, and when it does occur the quantity is very small. The principal part of the solids consists of salts and extractive bodies. In exceptional cases the fluid may be rich in proteid and may show a higher specific gravity.

In regard to the quantitative composition of the fluid from ovarial cysts we refer the reader to the work of OERUM.[1]

The Egg.

The small ova of man and mammals cannot, for evident reasons, be the subject of a searching chemical investigation. Up to the present time the eggs of birds, amphibians, and fishes have been investigated, but above all the hen's egg. We will here occupy ourselves with the constituents of this last.

The **yolk** of the hen's egg. In the so-called white yolk, which forms the *germ* with a process reaching to the centre of the yolk (*latebra*), and also a layer found between the yolk and yolk-membrane, we find *proteid, nuclein, lecithin*, and *potassium* (LIEBERMANN)[2]. The occurrence of glycogen is doubtful. The yolk-membrane consists of an albumoid similar in certain respects to keratin (LIEBERMANN).

The principal part of the yolk—the nutritive yolk or yellow—is a viscous, non-transparent, pale-yellow or orange-yellow alkaline emulsion of a mild taste. The yolk contains *vitellin, lecithin, cholesterin, fat, coloring matters*, traces of *neuridin* (BRIEGER),[3] *glucose* in very small quantities, and *mineral bodies*. The occurrence of cerebrin and of granules similar to starch (DARESTE)[4] has not been positively proved.

Ovovitellin. This body is generally considered as a globulin, but it resembles a nucleoalbumin more. The question as to what relationship other protein substances which, like the *aleuron-grains* of certain seeds and the so-called "*dotterplättchen*" of the eggs of certain fishes and amphibians, are related to ovovitellin, bear to this substance, is a question which requires further investigation.

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[1] Kemiske Studier over Ovariecystevædsker, etc. Koebenhavn, 1884. See also Maly's Jahresber., Bd. 14, S. 459.

[2] Pflüger's Arch., Bd. 43.

[3] Ueber Ptomaïne. Berlin, 1885.

[4] Compt. rend., Tome 72.

The ovovitellin which has been prepared from the yolk of eggs is not a pure albuminous body, but always contains lecithin. HOPPE-SEYLER found 25% lecithin in vitellin and also some pseudonuclein. The lecithin may be removed by boiling alcohol, but the vitellin is changed thereby, and it is therefore probable that the lecithin is chemically united with the vitellin (HOPPE-SEYLER).[1] BUNGE[2] prepared a pseudonuclein by digesting the yolk with gastric juice, and this pseudonuclein, according to him, is of great importance in the formation of the blood, and on these grounds he called it *hœmatogen*. This hæmatogen—whose composition is as follows: C 42.11, H 6.08, N 14.73, S 0.55, P 5.19, Fe 0.29, and O 31.05%—seems to be a decomposition product of vitellin.

Vitellin is similar to the globulins in that it is insoluble in water, but on the contrary soluble in dilute neutral-salt solutions (although the solution is not quite transparent). It is also soluble in hydrochloric acid of 1 p. m. and in very dilute solutions of alkalies or alkali carbonates. It is precipitated from its salt solution by diluting with water, and when allowed to stand some time in contact with water the vitellin is gradually changed, forming a substance more like the albuminates. The coagulation temperature for the solution containing salt (NaCl) lies between + 70° and 75° C. or, when heated very rapidly, at about + 80° C. Vitellin differs from the globulins in yielding pseudonuclein by pepsin digestion. It is not always or only in part precipitated by NaCl in substance.

The chief points in the preparation of ovovitellin are as follows: The yolk is thoroughly agitated with ether; the residue is dissolved in a 10% common-salt solution, filtered, and the vitellin precipitated by adding an abundance of water. The vitellin is now purified by repeatedly redissolving in dilute common-salt solutions and precipitating by water.

Ichthulin, which occurs in the eggs of the carp and other fishes, is, according to KOSSEL and WALTER,[3] an amorphous modification of the crystalline body *ichthidin*, which occurs in the eggs of the carp. Ichthulin is precipitated on diluting with water. It used to be considered as a vitellin. According to WALTER it yields a pseudonuclein on peptic digestion, and this pseudonuclein gives a reducing carbohydrate on boiling with sulphuric acid. Ichthulin has the following composition: C 53.42; H 7.63; N 15.63; O 22.19; S 0.41; P 0.43. It also contains iron.

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[1] Med. chem. Untersuch., S. 216.

[2] Zeitschr. f. physiol. Chem., Bd. 9.

[3] *Ibid.*, Bd. 15.

The yolk also contains, besides vitellin, *alkali-albuminate* and *albumin*.

The *fat* of the yolk of the egg is, according to LIEBERMANN,[1] a mixture of a solid and a liquid fat. The solid fat consists chiefly of tripalmitin with some stearin. On the saponification of the egg-oil LIEBERMANN obtained 40% oleic acid, 38.04% palmitic acid, and 15.21% stearic acid. The fat of the yolk of the egg contains less carbon than other fats, which may depend on the presence of mono- and diglycerides or on a quantity of fatty acid deficient in carbon (LIEBERMANN).

Lutein. Yellow or orange-red amorphous coloring matters occur in the yellow of the egg and in several other places in the animal organism; for instance, in the blood-serum and serous fluids, fatty tissues, milk-fat, *corpora lutea*, and in the fat-globules of the retina. These coloring matters, which also occur in the vegetable kingdom (THUDICHUM[2]), have been called *luteines* or *lipochromes*.

The luteines, which among themselves show somewhat different properties, are all soluble in alcohol, ether, and chloroform. They differ from the bile-pigment, bilirubin, in that they are not separated from their solution in chloroform by water containing alkali, and also in that they do not give the characteristic play of colors with nitric acid containing a little nitrous acid, but give a transient blue color, and lastly they give an absorption-spectrum of ordinarily two bands, of which one covers the line *F* and the other lies between the lines *F* and *G*. The luteines withstand the action of alkalies so that they are not changed when we remove the fats present by means of saponification.

Lutein has not been prepared pure. MALY[3] has found two pigments free from iron in the eggs of a water-spider (*maja squinado*), one a red, *vitellorubin*, and the other a yellow pigment, *vitellolutein*. Both of these pigments are colored blue by nitric acid containing nitrous acid, and beautifully green by concentrated sulphuric acid. The absorption-bands, especially of the vitellolutein, correspond very nearly with those of ovolutein.

The *mineral bodies* of the yolk of the egg consist, according to POLECK,[4] of 51.2–65.7 parts soda, 89.3–80.5 potash, 122.1–132.8

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[1] L. c.

[2] Centralbl. f. d. med. Wissensch., 1869, No. 1.

[3] Monatshefte f. Chem., Bd. 2.

[4] Cited from Gorup-Besanez, Lehrbuch d. physiol. Chem., 4. Aufl., S. 740.

lime, 20.7–21.1 magnesia, 14.5–11.90 iron oxide, 638.1–667.0 phosphoric acid, and 5.5–14.0 parts silicic acid in 1000 parts of the ash. We find phosphoric acid and lime the most abundant, and then potash, which is somewhat greater in quantity than the soda. These results are not, however, quite correct, first, because no dissolved phosphate occurs in the yolk (LIEBERMANN), and secondly, in burning, phosphoric and sulphuric acids are produced and these drive away the chlorine, which is not accounted for in the preceding analyses.

The yolk of the hen's egg weighs about 12–18 grms. The quantity of water and solids amounts, according to PARKES,[1] to 471.9 p. m. and 528.1 p. m. respectively. Among the solids he found 156.3 p. m. proteid, 3.53 p. m. soluble and 6.12 p. m. insoluble salts. The quantity of fat, according to PARKES, is 228.4 p. m., the lecithin, calculated from the amount of phosphorus in the organic substance in the alcohol-ether extract, was 107.2 p. m., and the cholesterin 17.5 p.m.

The **white of the egg** is a faint-yellowish albuminous fluid enclosed in a framework of thin membranes; and this fluid is in itself very liquid, but seems viscous because of the presence of these fine membranes. That substance which forms the membranes, and of which the *chalaza* consists, seems to be a body nearly related to horn substances (LIEBERMANN[2]).

The white of the egg has a specific gravity of 1.045 and always has an alkaline reaction. It contains 850–880 p. m. water, 100–130 p. m. proteid bodies, and 7 p. m. salts. Among the extractive bodies LEHMANN found a fermentable *variety of sugar* which amounted to 5 p. m. or, according to MEISSNER, 80 p. m. of the solids.[3] Besides these, we find in the white of the egg traces of fats, soaps, lecithin, and cholesterin.

The white of the egg during incubation becomes transparent on boiling and acts in many respects like alkali-albuminate. This albumin TARCHANOFF[4] called "*tatalbumin.*"

The albuminous bodies of the white of the egg belong partly to the globulin and partly to the albumin group. Besides these, the white of the egg contains a mucoid substance.

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[1] Hoppe-Seyler, Med. chem. Untersuch., Heft 2, S. 209

[2] L. c.

[3] Cited from Gorup-Besanez, Lehrbuch, 4. Aufl., S. 739.

[4] Pflüger's Arch., Bdd. 31, 33, and 39.

The *ovglobulin* is, according to DILLNER,[1] closely related to serglobulin. On diluting the white of the egg with water it partly separates. It is also precipitated by magnesium sulphate. The quantity of globulins in the white of the egg is on an average 6.67 p. m., or about 67 p. m. of the total proteids. According to CORIN and BERARD,[2] we have two globulins in the white of thė egg, one coagulating at + 57.5° C., and the other at + 67° C.

Ovalbumin, or the albumin of the white of the egg. Ovalbumin was first obtained in a crystalline form by HOFMEISTER[3], by allowing its solution in a half-saturated ammonium-sulphate solution to evaporate very slowly. This crystalline ovalbumin is later further studied by GABRIEL,[4] BONDZYNSKI and ZOJA,[5] and the two last-mentioned investigators were able, by fractional crystallization, to show that ovalbumin was probably a mixture of several albumins of about the same elementary composition but with somewhat different coagulation-temperature, solubility, and specific rotation. In the main these results are in accord with the views of many other investigators, such as GAUTIER,[6] BÉCHAMP,[7] CORIN and BERARD,[8] on the occurrence of several albumins, but in details they do not agree very well. According to GAUTIER and BÉCHAMP ovalbumin is a mixture of two albumins with the coagulation-temperature of 60–63° and 71–74° C. respectively, while according to CORIN and BERARD it is a mixture of three albumins with the coagulation-temperature of 67, 72, and 82° C., respectively. According to BONDZYNSKI and ZOJA the portion which dissolves with difficulty coagulates at 64.5°, while the readily soluble portion coagulates at 55.5–56° C. The elementary composition of ovalbumin has not been positively established. BONDZYNSKI and ZOJA found C 52.07–52.44, H 6.95–7.26, N. 15.11–15.58, and S 1.61–1.70% for four different fractions, which agree well with the results of the author, namely, C 52.25, H 6.90, N 15.25, S 1.67–1.93%. HOFMEISTER,[9] on the contrary, found higher figures, 53.28%, for the carbon and lower, 15.0 and 1.09%, for the nitrogen and sulphur respectively. The specific rotation was determined by STARKE[1] as α (D) = − 38°. BONDZYNSKI and ZOJA found 25.8–26.2°, 29.16°, 34.18°, and 42.54° for various fractions. Ovalbumin has the properties of the albumins in general, but differs from seralbumin in the following: Its specific rotation is lower. It is quickly rendered insoluble by alcohol. It is precipitated by a sufficient quantity of hydrochloric acid, but dissolves with greater difficulty than seralbumin in an excess of the acid. Ovalbumin in solution, when introduced into the blood-circulation, passes into the urine, which is not the case with seralbumin.

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[1] Upsala Läkarefs. Förh., Bd. 20; also Maly's Jahresber., Bd. 15, S. 31.

[2] Travaux du laboratoire de l'Université de Liège, Tome 2; also Maly's Jahresber., Bd. 18, S. 13.

[3] Zeitschr. f. physiol. Chem., Bdd. 14 and 16.

[4] *Ibid.*, Bd. 15.

[5] *Ibid.*, Bd. 19.

[6] Bull. de la soc. chim., Tome 14.

[7] *Ibid.*, Tome 21.

[8] L. c.

[9] Zeitschr. f. physiol. Chem., Bd. 16.

Ovalbumin, or, more correctly, the mixture of albumins, may be obtained, according to STARKE, by precipitating the globulins by $MgSO_4$ at 20° C. and saturating the filtrate with Na_2SO_4 at the same temperature. The ovalbumin which separates is filtered, pressed, dissolved in water, and freed from salts by dialysis. The dialyzed solution is then evaporated in a vacuum or at 40°–50° C. If precipitated with alcohol, albumin becomes quickly insoluble.

To prepare crystallized ovalbumin mix the white of egg, previously beaten and separated from the foam, with an equal volume of a saturated solution of ammonium sulphate, filter off the globulin, and allow the filtrate to evaporate slowly in not too thin layers at the temperature of the room. The mass, which separates after a time, is dissolved in water, treated with ammonium sulphate solution until a cloudiness commences, and then allowed to stand. After repeated recrystallizations the mass is treated either with alcohol, which makes the crystals insoluble, or they are dissolved in water and purified by dialysis. The albumin does not crystallize from this solution on spontaneous evaporation.

Ovomucoid. This substance, first observed by NEUMEISTER[2] and considered by him as pseudo-peptone and then later studied by SALKOWSKI,[3] is, according to C. TH. MÖRNER,[4] a mucoid with 12.65% nitrogen and 2.20% sulphur. On boiling with dilute mineral acids it yields a reducing substance. Ovomucoid exists to a great extent in hens' eggs, the solids of which, in round numbers, contain 10%.

A solution of ovomucoid is not precipitated by mineral acids nor by organic acids, with the exception of phosphotungstic acid and tannic acid. It is not precipitated by metallic salts, but basic lead acetate and ammonia give a precipitate. Ovomucoid is precipitated by alcohol, but sodium chloride, sodium sulphate, and magnesium sulphate give no precipitates either at the ordinary temperature nor when added to saturation at 30° C. Its solutions are not precipitated by an equal volume of a saturated solution of ammonium sulphate, but are precipitated on adding more salt thereto. The substance is not precipitated on boiling, but the part which has become insoluble in cold water and then dried is precipitated when dissolved in boiling water.

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[1] Upsala Läkarefs Förh., Bd. 16; also Maly's Jahresber., Bd. 11, S. 17.

[2] Zur Physiologie der Eiweissresorption, etc. Zeitschr. f. Biologie., Bd., 27.

[3] Centrabl. f. d. med. Wissensch. 1893.

[4] Zeitschr. f. physiol. Chem. Bd. 18.

Ovomucoid may be prepared by removing all the proteids by boiling with the addition of acetic acid, and then concentrating the filtrate and precipitating with alcohol. The substance is purified by repeated solution in water and precipitating with alcohol.

The *mineral bodies* of the white of the egg have been analyzed by POLECK and WEBER.[1] They found in 1000 parts of the ash: 276.6–284.5 grms. potash, 235.6–329.3 soda, 17.4–29 lime, 16–31.7 magnesia, 4.4–5.5 iron oxide, 238.4–285.6 chlorine, 31.6–48.3 phosphoric acid (P_2O_5), 13.2–26.3 sulphuric acid, 2.8–20.4 silicic acid, and 96.7–116 grms. carbon dioxide. Traces of fluorine have also been found (NICKLÉS[2]). The ash of the white of the egg contains, as compared with the yolk, a greater amount of chlorine and alkalies, and a smaller amount of lime, phosphoric acid, and iron.

The Shell-membrane and the Egg-shell. The shell-membrane consists, as above stated (page 49), of a keratin substance. The shell contains very little organic substance, 36–65 p. m. The chief mass, more than 900 p. m., consists of calcium carbonate; besides this there are very small amounts of magnesium carbonate and earthy phosphates.

The different *coloring* of birds' eggs depends upon several different coloring matters. Among these we find a red or reddish-brown pigment called "*oorodein*" by SORBY,[3] which is perhaps identical with hæmatoporphyrin. The green or blue coloring matter, SORBY'S *oocyan*, seems, according to LIEBERMANN,[4] and KRUKENBERG,[5] to be partly *biliverdin* and partly a blue *derivative of the bile-pigments.*

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[1] Cited from Hoppe Seyler's Physiol. Chem., S. 778.

[2] Comp. rend., Tome 48.

[3] Cited from Krukenberg, Verh. d. phys.-chem. Gesellsch. in Würzburg, Bd. 17.

[4] Ber. d. deutsch. chem. Gesellsch., Bd. 11.

[5] L. c.

The eggs of birds have a space at their blunt end filled with gas; this gas contains on an average 18.9–19.9 per cent oxygen (HUFNER).[1]

The weight of a hen's egg varies between 40–60 grammes and may weigh sometimes 70 grms. The shell and shell-membrane together, when carefully cleaned, but still in the moist state, weigh 5–8 grms. The yolk weighs 12–18 and the white 23–34, or about double.

The white of the egg of cartilaginous and bony fishes contains only traces of true albumin, and the cover of the frog's egg consists, according to GIACOSA,[2] of mucin. The crystalline formations (*yolk-spherules* or *dotterplättchen*) which have been observed in the egg of the tortoise, frog, ray, shark, and other fishes, and which are described by VALENCIENNES and FREMY[3] under the names *emydin*, *ichthin*, *ichthidin*, and *ichthulin*, seem, as above stated in connection with ichthulin, to consist chiefly of phosphoglycoproteids. The egg of the river-crab and the lobster contain the same pigment as the shell of the animal. This pigment, called *cyanocrystallin*, becomse red on boiling in water.

In fossil eggs (of APTENODYTES, PELECANUS, and HALLÆUS) in old guano deposits a yellowish-white, silky, laminated combination has been found which is called *guanovulit*, $(NH_4)_2SO_4 + 2K_2SO_4 + 3KHSO_4 + 4H_2O$, and which is easily soluble in water, but is insoluble in alcohol and ether.

Those eggs which develop outside of the mother-organism must contain all the elements necessary for the young animals. One finds, therefore, in the yolk and white of the egg an abundant quantity of albuminous bodies of different kinds, and especially a phosphorized proteid in the yolk. Further, we also find lecithin in the yolk, which seems habitually to occur in the developing cell. The occurrence of glycogen is doubtful, and the carbohydrates are perhaps represented by a very small amount of glucose and ovomucoids. On the contrary, the egg contains a large proportion of fat, which doubtless is an important source of nutrition and respiration for the embryo. The cholesterin and the lutein can hardly have a direct influence on the development of the embryo. The egg also seems to contain the mineral bodies necessary for the development of the young animal. The lack of phosphoric acid is compensated by an abundant amount of phosphorized organic substance, and the nucleoalbumin containing iron, from which the hæmatogen (see page 411) is formed, is doubtless, as BUNGE claims, of great importance in the formation of the hæmoglobin containing iron. The silicic acid necessary for the development of the feathers is also found in the egg.

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[1] Du Bois-Reymond's Arch., 1892.

[2] Zeitschr. f. physiol. Chem., Bd. 7.

[3] Cited from Hoppe-Seyler's Physiol. Chem., S. 77.

During the period of incubation the egg loses weight, chiefly due to loss of water. The quantity of solids, especially the fat and the proteids, diminishes and the egg gives off not only carbon dioxide, but also, as LIEBERMANN[1] has shown, nitrogen or a nitrogenous substance. The loss is compensated by the absorption of oxygen, and it is found that during incubation a respiratory exchange of gas takes place. While the quantity of dry substance in the egg during this period always decreases, the quantity of mineral bodies, proteid, and fat always increases in the embryo. The increase in the amount of fat in the embryo depends, according to LIEBERMANN, in great part upon a taking up of the nutritive yolk in the abdominal cavity. The weight of the shell and the quantity of lime-salts contained therein remains unchanged during incubation. The yolk and white together contain the necessary quantity of lime for development.

The most complete and careful chemical investigation on the development of the embryo of the hen has been made by LIEBERMANN. From his researches we may quote the following: In the earlier stages of the development, tissues very rich in water are formed, but on the continuation of the development the quantity of water decreases. The absolute quantity of bodies soluble in water increases with the development, while their relative quantities, as compared to the other solids, continually decreases. The quantity of bodies soluble in alcohol quickly increases. A specially important increase is noticed in the fat, whose quantity is not very great even on the fourteenth day, but after that it becomes considerable. The quantity of albuminous bodies and albuminoids insoluble in water grows continually and regularly in such a way that their absolute quantity increases while their relative quantity remains nearly unchanged. LIEBERMANN found no gelatin in the embryo of the hen. The embryo does not contain any gelatin-forming substance until the tenth day, and from the fourteenth day on it contains a body which when boiled with water gives a substance similar to chondrin. A body similar to mucin occurs in the embryo when about six days old, but then disappears. The quantity of hæmoglobin shows a continual increase compared to the weight of the body. LIEBERMANN found that the relationship of the hæmoglobin to the bodily weight was 1:728 on the eleventh day and 1:421 on the twenty-first day.

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[1] Pflüger's Arch., Bd. 43.

The tissue of the **placenta** has not thus far been the subject of detailed chemical investigation. In the edges of the placenta of bitches and of cats a crystallizable orange-colored pigment (bilirubin ?) has been found, and also a green amorphous pigment, Meckel's *hæmatochlorin*, which is considered as biliverdin by Etti.[1] Preyer[2] questions the identity of these pigments with biliverdin.

From the cotyledons of the placenta in ruminants a white or faint rose-colored creamy fluid, the *uterine milk*, can be obtained by pressure. It is alkaline in reaction, but becomes acid quickly. Its specific gravity is 1.033–1.040. It contains as form-elements fat-globules, small granules, and epithelium-cells. We have found 81.2–120.9 p. m. solids, 61.2–105.6 p. m. proteid, about 10 p. m. fat, and 3.7–8.2 p. m. ash in the uterine milk.

The fluid occurring in the so-called GRAPE-MOLE (*mola racemosa*) has a low specific gravity, 1.009–1.012, and contains 19.4–26.3 p. m. solids with 9–10 p. m. protein bodies and 6–7 p. m. ash.

The **amniotic fluid** is in women thin, whitish, or pale yellow; sometimes it is somewhat yellowish brown and cloudy. White flakes separate. The form-elements are *mucus-corpuscles, epithelium-cells*, *fat-drops,* and *lanugo hair*. The odor is stale, the reaction neutral or faintly alkaline. The specific gravity is 1.002-1.028.

The amniotic fluid contains the constituents of ordinary transudations. The amount of solids at birth is hardly 20 p. m. In the earlier stages of pregnancy the fluid contains more solids, especially proteids. Among the albuminous bodies, Weyl[3] found one substance similar to *vitellin*, and with great probability also *seralbumin,* besides small quantities of *mucin*. *Glucose* is regularly found in the amniotic fluid of cows, but not in human beings. On the contrary, the human amniotic fluid contains some *urea* and *allantoin*. The quantity of these may be increased in hydramnion (Prochownick,[4] Harnack),[5] which depends on an increased secretion by the kidneys and skin of the fœtus. Creatin and lactates are questionable constituents of the amniotic fluid. The quantity of urea in the amniotic fluid is, according to Prochownick, 0.16 p. m. In the fluid in hydramnion, Prochownick and Harnack found respectively 0.34 and 0.48 p. m. urea. The chief mass of the solids consists of salts. The quantity of chlorides (NaCl) is 5.7–6.6 p. m.

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[1] Maly's Jahresber., Bd. 2, S. 287.

[2] Die Blutkrystalle (Jena, 1871), S. 189; Du Bois-Reymond's and Reichert's Arch., 1876.

[3] *Ibid.*

[4] Arch. f. Gynäk., Bd. 11; also Maly's Jahresber., Bd. 7, S. 155.

[5] Berlin klin. Wochenschr., 1888, No. 41.

CHAPTER XIV.

MILK.

THE chemical constituents of the *mammary glands* have been little studied. The protoplasm of the cells is rich in proteid, which consists in great part of casein or a substance nearly related. If all the milk is removed from the mammary gland by thorough washing, the cells still contain a large quantity of proteids which swell up to a slimy, ropy, or fibrous mass when very dilute alkali (1–2 p. m. KOH) is added. These proteids consist mainly of nucleoproteid, which is gradually changed by the action of the alkali. This nucleoproteid gives a reducing substance on boiling with dilute acids. If the mammary gland is boiled with water, the protoplasm of the cell is decomposed and a nucleoproteid passes into solution, which may be precipitated by the addition of acetic acid, and which is characterized by its greater insolubility in acetic acid, compared with casein. This nucleoproteid, which may well be considered as a protoplasm-nucleoproteid changed by heat, also gives on boiling with dilute mineral acids a reducing substance whose nature is not known. The relation this nucleoproteid bears to lactose or the mother-substance of the same has not been determined. According to BERT,[1] the secreting glands contain a body which on boiling with dilute mineral acids yields a reducing substance. Such a substance, which acts as a step towards the formation of lactose, has also been observed by THIERFELDER.[2] Fat seems to be a never-failing constituent of the cell, at least in the secreting gland, and this fat ma be observed in the protoplasm as large or small globules similar milk-globules. The extractive bodies of the mammary glands h been little investigated, but among them we find considerable amounts of xanthin bases.

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[1] Compt. rend., Tome 98.

[2] Pflüger's Arch., Bd. 32, and Maly's Jahresber., Bd. 18, S. 156.

As human milk and milk of animals are essentially of the same constitution, it seems best to speak first of the one most thoroughly investigated, namely, cow's milk, and then of the essential properties of the remaining important varieties of milk.

Cow's Milk.

Cow's milk forms, as all milks do, an emulsion which consists of very finely divided fat suspended in a solution consisting chiefly of proteid bodies, milk-sugar, and salts. Milk is non-transparent, white, whitish yellow, or in thin layers somewhat bluish white, of a faint, insipid odor and mild, faintly sweetish taste. The specific gravity is 1.028 to 1.0345 at + 15° C.

The reaction of perfectly fresh milk is generally amphoteric. The extent of the acid and alkaline part of this amphoteric reaction has been determined by different investigators, especially THÖRNER,[1] SEBELIN,[2] and COURANT.[3] The results are different on using different indicators, and also the milk from various animals, as well as at different times during the lactation period, differs somewhat. The first and last portions of the same milking have a different reaction. COURANT has determined the alkaline part by $\frac{N}{10}$ sulphuric acid, using blue lacmoid as indicator and the acid part by $\frac{N}{10}$ caustic soda, using phenolphthalein as indicator. He found, as average for the first and last portions of the milking of twenty cows, that 100 cc. milk had the same alkaline reaction for blue lacmoid as 41 cc. $\frac{N}{10}$ caustic soda, and the same acid reaction for phenolphthalein as 19.5 cc. $\frac{N}{10}$ sulphuric acid.

Milk gradually changes when exposed to the air, and its reaction becomes more and more acid. This depends on a gradual transformation of the milk-sugar into lactic acid, caused by micro-organisms.

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[1] Chem. Ztg., Bd. 16, S. 1469.

[2] *Ibid.*, Bd. 16, S. 597.

[3] Ueber die Reaktion der Kuh- und Frauenmilch, etc. Inaug.-Diss. Bonn, 1891; also Pflüger's Arch., Bd. 50.

Entirely fresh amphoteric milk does not coagulate on boiling, but forms a skin consisting of coagulated casein and lime-salts, which rapidly re-forms after being removed. Even after passing a current of carbon dioxide through the fresh milk it does not coagulate on boiling. In proportion as the formation of lactic acid advances this behavior changes, and soon a stage is reached when the milk, which has previously had carbon dioxide passed through it, coagulates on boiling. At a second stage it coagulates alone on heating; then it coagulates by passing carbon dioxide alone without boiling; and lastly, when the formation of lactic acid is sufficient, it coagulates spontaneously at the ordinary temperature, forming a solid mass. It may also happen, especially in the warmth, that the casein-clot contracts and a yellowish or yellowish-green acid liquid (acid whey) separates.

If the drawn is sterilized by heating and contact with micro-organisms prevented, the formation of lactic acid may be entirely stopped. The formation of acid may also be prevented, at least for some time, by many antiseptics, such as salicylic acid (1:5000), thymol, boracic acid, and other bodies.

If freshly drawn amphoteric milk is treated with rennet, it coagulates quickly, especially at the temperature of the body, to a solid mass (curd) from which a yellowish fluid (sweet whey) is gradually pressed out. This coagulation occurs without any change in the reaction of the milk, and therefore it is distinct from the acid coagulation.

Milk sometimes undergoes a peculiar kind of coagulation, being converted into a thick, ropy, slimy mass (thick milk). This conversion depends, according to SCHMIDT-MÜLHEIM,[1] upon a peculiar change in which the milk-sugar is made to undergo a slimy transformation. This transformation is caused by a special organized ferment.

In cow's milk we find as form-elements a few colostrum corpuscles (see Colostrum) and a few pale nucleated cells. The number of these form-elements is very small compared with the immense amount of the most essential form-constituents, the milk-globules.

The Milk-globules. These consist of extremely small drops of fat whose number is, according to WOLL,[2] 1.03–5.75 million in

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[1] Pflüger's Arch., Bd. 27.

[2] On the Conditions influencing the Number and Size of Fat-globules in Cow's Milk. Wisconsin Expt. Station, Vol. 6, 1892.

1 c.mm., and whose diameter is 0.0024–0.0046 mm. and 0.0037 mm. as average for animals of different races. It is unquestionable that the milk-globules contain fat, and we consider it as positive that all the milk-fat exists in them. Another and disputed question is whether the milk-globules consist entirely of fat or whether they also contain proteid.

According to the observations of ASCHERSON,[1] drops of fat, when dropped in an alkaline proteid solution, are covered with a fine albuminous coat, a so-called *haptogen-membrane*. As milk on shaking with ether does not give up its fat, or only very slowly, in the presence of a great excess of ether, and as this takes place very readily after the addition of acids or alkalies, which dissolve proteids, it was formerly thought that the fat-globules of the milk were enveloped in a proteid coat. A true membrane has not been detected; and since, when no means of dissolving the proteid is resorted to—for example, when the milk is precipitated by carbon dioxide after the addition of very little acetic acid, or when it is coagulated by rennet—the fat can be very easily extracted by ether, the theory of a special albuminous membrane for the fat-globules has been generally abandoned. The observations of QUINCKE[2] on the behavior of the fat-globules in an emulsion prepared with gum have led, at the present time, to the conclusion that each fat-globule in the milk is surrounded by a stratum of casein solution by means of molecular attraction, and this prevents the globules from uniting with each other. Everything that changes the physical property of the casein in the milk or precipitates it must necessarily help the solution of the fat in ether, and it is in this way that the alkalies, acids, and rennet work.

If we accept this view, which requires further proof, we must not overlook the fact that the fat-globules remain unchanged when the milk under agitation is coagulated with rennet. In this case we find an immense number of unchanged milk-globules in the whey, and if we wish to admit of a stratum of proteids around the fat-globules proceeding from the molecular attraction, we must not consider that it is entirely due to casein, but to proteid in general.

If the fat-globules are filtered off and washed on a filter, we always obtain (RADENHAUSEN and DANILEWSKY)[3] after their treatment with ether a residue consisting of proteid. From this behavior the deduction has been made that the fat-globules, even though they have no real membrane, consist, nevertheless, of fat and proteid. The extreme difficulty of completely removing the albuminous bodies of the milk by washing the fat on the filter renders it necessary to exercise great caution in drawing a conclusion. The question as to the composition of the milk-globules, and especially as to the possible amount of proteid, cannot be decided at present.

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[1] Arch. f. Anat. u. Physiol.. 1840.

[2] Pflüger's Arch., Bd. 19.

[3] Forschungen auf dem Gebeite der Viehhaltung (Bremen, 1880), Heft 9.

The *milk-fat* has a rather variable specific gravity, which according to Bohr[1] is 0.949–0.996 at + 15° C. The milk-fat, which is obtained under the name of butter, consists in great part of the neutral fats *palmitin, olein,* and *stearin.* Besides these it contains, as triglycerides, *myristic acid,* small quantities of *butyric acid* and *caproic acid,* traces of *caprylic acid, capric acids, lauric acid,* and *arachidic acids.* Butter which has been exposed to the action of sunlight contains also formic acid (Duclaux). Milk-fat also contains a small quantity of *lecithin* and *cholesterin,* also a yellow *coloring matter.* The quantity of volatile fatty acids in butter is, according to Duclaux,[2] on an average about 70 p. m., of which 37–51 p. m. is butyric acid and 20–33 p. m. is caproic acid. The non-volatile fat consists of $\frac{3}{10}$ to $\frac{4}{10}$ olein, and the remainder of a mixture of palmitin and stearin.

According to other investigators milk-fat has a different composition. Koefoed[3] found in butter from Jütland besides oleic acid two other acids not belonging to the series $C_nH_{2n}O_2$, having the formulæ $C_{15}H_{28}O_4$ and (probably) $C_{20}H_{34}O_5$.

In 100 parts fatty acids he found 66 parts acids of the series $C_nH_{2n}O_2$, namely, 2 stearic acid, 28 palmitic acid, 22 myristic acid, 8 lauric acid, 1.5 butyric acid, 2 caproic acid, 2 capric acid, and 0.5 caprylic acid. According to Wanklyn[4] butter does not contain any palmitic acid. It contains instead an acid called by him *aldepalmitic acid,* with the formula $(C_{16}H_{30}O_2)_n$, and not belonging to the oleic acid series. The relative quantities of the different fatty acids do not seem to be constant, and they differ at various times during lactation.

The quantity of volatile fatty acids in butter-fat is of great practical importance in the methods for detecting the presence of foreign fats in butter. This detection is performed generally according to Reichert's process based on Hehner and Angell's method. The fat is saponified with alcoholic potash and the alcohol evaporated. The soaps are dissolved in water, and then distilled with an excess of phosphoric acid. The quantity of volatile fatty acids in the distillate is determined by titration with decinormal alkali. With butter of proper composition 2.5 grms. should yield a distillate requiring 14–13 c.c. for neutralization, and at least not less than 12 c.c. of the decinormal alkali. In proportion as the butter contains a greater quantity of foreign fats the quantity of alkali required becomes smaller. We cannot here describe in detail the different modifications of this process as well as the newer methods.

The **milk-plasma,** or that fluid in which the fat-globules are suspended, contains at least three different albuminous bodies, *casein, lactoglobulin,* and *lactalbumin,* and two carbohydrates, of which only one, the *milk-sugar*, is of great importance. The milk-plasma also contains extractive bodies, traces of *urea, creatin, creatinin, hypoxanthin (?), lecithin, cholesterin, citric acid* (SOXHLET and HENKEL),[1] and lastly also *mineral bodies* and *gases*.

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[1] Studier over Maelk. Kjöbenhavn, 1880, and Maly's Jahresber., Bd. 10, S. 182.

[2] Compt. rend., Tome 104.

[3] Bull. de l'Acad. Roy. Danoise, 1891.

[4] Chem. News, Vol. 63.

Casein. This protein substance, which thus far has been detected positively only in milk, belongs to the nucleoalbumins, and differs from the albuminates by its containing phosporus and by its behavior with the rennet enzyme. Casein from cow's milk has the following composition: C 53.0, H 7.0, N 15.7, S 0.8, P 0.85, and O 22.65%. Its specific rotation is, according to HOPPE-SEYLER,[2] somewhat variable; in neutral solution it is α (D) = − 80°. The question whether the casein from different varieties of milk is identical or whether there are several different caseins has not been positively determined.

Casein when dry appears like a fine white powder which, after heating to 100° C. or somewhat above, shows the properties and solubilities of freshly precipitated, still-moist casein. Casein is only slightly soluble in water or in neutral-salt solutions. According to ARTHUS[3] it is rather easily soluble in a 1% solution of sodium fluoride, ammonium, or potassium oxalate. It acts like a rather strong acid, dissolves readily in water on the addition of very little alkali, forming a neutral or acid liquid, and lastly it dissolves in water in the presence of calcium carbonate, from which it expels the carbon dioxide. If casein is dissolved in lime-water and this solution carefully treated with very dilute phosphoric acid until it is neutral in reaction, the casein appears to remain in solution, but is probably only swollen as in milk, and the liquid contains at the same time a large quantity of calcium phosphate without any precipitate or any visible suspended particles. The casein solutions containing lime are opalescent and have on warming the appearance of milk deficient in fat. Therefore it is not impossible that the white color of the milk is due partly to the casein and calcium phosphate. SÖLDNER has prepared two calcium combinations of casein with 1.55 and 2.36% CaO, and these combinations are designated di- and tricalcium casein by COURANT.[4]

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[1] Cited from F. Söldner, Die Salze der Milch, etc. Landwirthsch. Versuchsstation, Bd. 35. Separatabzug, S. 18.

[2] Handb. d. physiol. u. pathol. chem. Analyse, 6. Aufl., S. 259.

[3] Thèses presentées à la faculté des sciences de Paris, 1893.

[4] L. c.

Casein solutions do not coagulate on boiling, but are covered, like milk, with a skin. They are precipitated by very little acid, but the presence of neutral salts retards the precipitation. A casein solution containing salt or ordinary milk requires, therefore, more acid for precipitation than a salt-free solution of casein of the same concentration. The precipitated casein dissolves very easily again in a small excess of the acid, but less easily in an excess of acetic acid. The acid solutions are precipitated by mineral acids in excess. Casein is precipitated from neutral solutions or from milk by common salt or magnesium sulphate in substance without changing its properties. Metallic salts, such as copper sulphate, completely precipitate the casein from neutral solutions.

The property which is the most characteristic of casein is that it coagulates with rennet in the presence of a sufficiently great amount of lime-salts. In solutions free from lime-salts the casein does not coagulate with rennet; but it is changed so that the solution (even if the enzyme is destroyed by heating) yields a coagulated mass, having the properties of curd, if lime-salts are added. The rennet enzyme, rennin, has therefore an action on casein even in the absence of lime-salts, and these last are only necessary for the coagulation or the separation of the curd. This fact, which was first proved by the AUTHOR,[1] has lately been confirmed by ARTHUS and PAGES.[2] PETERS[3] claims to have found that paracasein, when dissolved in lime-water, may be repeatedly coagulated by rennet. According to PETERS rennet also coagulates alkali albuminate, as also vegetable proteid bodies precipitated by acids (wheat and peas) when dissolved in lime-water. Several enzymes existing in the planet kingdom also have the same action as rennet.

The curd formed on the coagulation of milk contains large quantities of calcium phosphate. According to SOXHLET and SÖLDNER,[4] the soluble lime-salts are only of essential importance in coagulation, while the calcium phosphate is without importance. According to COURANT[5] the calcium casein on coagulation m carry down with it, if the solution contains dicalcium phosphate, part of this as tricalcium phosphate, leaving monocalcium phosphate in the solution. The chemical processes which take place in the rennet coagulation have not been thoroughly investigated; still several observations seem to show that casein splits partly into a difficultly soluble body, *paracasein* or *curd*, whose composition closely resembles that of casein and which forms the chief product, and partly into an easily soluble substance, similar to albumose, *whey-proteid*, which is deficient in carbon and nitrogen (50.3% C and 13.2% N, KÖSTNER[1]) and which is produced in very small quantities. Paracasein[2] is not further changed by the rennet enzyme, and it has not the same property of holding calcium phosphate in solution as casein has.

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[1] Maly's Jahresber., Bdd. 2 and 4; also Hammarsten, Zur Kenntniss d Kaseïns und der Wirkung des Labfermentes. Nova Acta Reg. Soc. Scie Upsala, 1877. Festschrift.

[2] Arch. de Physiol. (5), Tome 2, and Mem. Soc. biol., Tome 43.

[3] Unters. über das Lab und die Labähnlichen Fermente. Rostock, 1894.

[4] L. c.

[5] L. c.

In the digestion of casein with pepsin hydrochloric acid pseudonuclein is split off. The quantity of pseudonuclein split off is, according to MORACZEWSKI,[3] very considerable, from 1.29 to 21.10% of the digested casein. SALKOWSKI and HAHN[4] and SIEBELIEN[5] have also found with MORACZEWSKI that the quantity of pseudonuclein split off in the peptic digestion of casein is very variable. SEBELIEN as well as WILLDENOW and MORACZEWSKI could not bring all the pseudonuclein in solution by continuous digestion. The quantity of phosphorus in the pseudonuclein also varies between 0.88 and 6.86%, and of the casein phosphorus varying quantities, 6 to 60%, were obtained in the pseudonuclein. All the phosphorus of the casein was never obtained as pseudonuclein, and MORACZEWSKI draws the conclusion from his investigations that the pseudonuclein from the beginning does not contain all the phosphorus of the casein.

Casein may be prepared in the following way: The milk is diluted with 4 vols. water and the mixture treated with acetic acid to 0.75 to 1 p. m. Casein thus obtained is purified by repeated solution in water with the aid of the smallest quantity of alkali possible, by filtrating and reprecipitating with acetic acid, and thoroughly washing with water. Most of the milk-fat is retained by the filter on the first filtration, and the casein contaminated with traces of fat is purified by treating with alcohol and ether.

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[1] See Maly's Jahresber., Bd. 11, S. 14.

[2] It has been recently proposed to designate the ordinary casein as caseinogen, and the curd as casein. Although such a proposition is theoretically correct, it leads in practice to confusion. On this account the author calls the curd paracasein, according to Schulze and Röse (Landwirthsch. Versuchsstat., Bd. 31).

[3] Zeitschr. f. physiol. Chem., Bd. 20.

[4] Pflüger's Arch., Bd. 50.

[5] Zeitschr. f. physiol. Chem., Bd. 20.

Lactoglobulin was obtained by SEBELIEN[1] from cow's milk by saturating it with NaCl in substance (which precipitated the casein), and saturating the filtrate with magnesium sulphate. As far as it has been investigated it had the properties of serglobulin, with which it is perhaps identical.

Lactalbumin was first prepared in a pure state from milk by SEBELIEN.[2] Its composition is, according to SEBELIEN, C 52.19, H 7.18, N 15.77, S 1.73, O 23.13%. Lactalbumin has the properties of the albumins. It coagulates, according to the concentration and the amount of salt in solution, at + 72° to 84° C. It is similar to seralbumin, but differs from it in having a considerably lower specific rotatory power: α (D) = − 37°.

The principle of the preparation of lactalbumin is the same as for the preparation of seralbumin from serum. The casein and the globulin are removed by $MgSO_4$ in substance and the filtrate treated as previously stated (page 122).

The occurrence of other albuminous bodies, such as *albumoses* and *peptones*, in milk has not been positively proved. These bodies are easily produced as laboration products from the other proteids of the milk. Such a laboration product is MILLON'S and COMAILLE'S *lactoprotein*, which is a mixture of a little casein with changed albumin, and albumose,[3] which is formed by the chemical operations.

Milk-sugar, LACTOSE, $C_{12}H_{22}O_{11} + H_2O$. This sugar with the absorption of water can be split into two glucoses, *dextrose* and *galactose*. It yields mucic acid by the action of dilute nitric acid, besides other organic acids. Levulinic acid is formed, besides formic acid and humin substances, by the stronger action of acids. By the action of alkalies amongst other products we find lactic acid and pyrocatechin.

Milk-sugar occurs, as a rule, only in milk, but it has also been found in the urine of pregnant women on stagnation of milk. According to the statements of PAPPEL and RICHMOND[4] the milk of the Egyptian buffalo does not contain milk-sugar, but a sugar which they call *tewfikose*.

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[1] Zeitschr. f. physiol. Chem., Bd. 9.

[2] L. c.

[3] See Hammarsten, Ueber das Laktoprotein. Nord. med. Arkiv., Bd. 8, 9; also Maly's Jahresber., Bd. 6, S. 13.

urn. Chem. Soc., London, 1894, p. 754.

Milk-sugar occurs ordinarily as colorless rhombic crystals with 1 mol. of water of crystallization, which is driven off by slowly heating to 100° C., but more easily at 130–140° C. At 170° to 180° C. it is converted into a brown amorphous mass, lactocaramel, $C_6H_{10}O_5$. Milk-sugar dissolves in 6 parts cold and in 2.5 parts boiling water; it has a faint sweetish taste. It does not dissolve in ether or absolute alcohol. Its solutions are dextrogyrate. The rotatory power, which on heating the solution to 100° C. becomes constant, is $\alpha\ (D) = + 52.5°$. Milk-sugar combines with bases; the alkali combinations are insoluble in alcohol.

Milk-sugar is not fermentable with pure yeast. It undergoes, on the contrary, alcoholic fermentation by the action of certain schizomycetes, and lactic acid is produced thereby. The preparation of milk-wine, "*kumyss*," from mare's milk and "*kephir*" from cow's milk is based upon this fact. Micro-organisms produce a lactic-acid fermentation in lactose, and this explains the ordinary souring of milk.

Lactose responds to the reactions of grape-sugar, such as MOORE'S or TROMMER'S, and the bismuth test, which will all be described in Chapter XV on the urine. It also reduces mercuric oxide in alkaline solutions. After warming with phenylhydrazin acetate it gives on cooling a yellow crystalline precipitate of phenyllactosazon, $C_{24}H_{32}N_4O_9$. It differs from cane-sugar by giving positive reactions with MOORE'S or TROMMER'S and the bismuth test, and also that it does not darken when heated with anhydrous oxalic acid to 100° C. It differs from grape-sugar and maltose by its solubility and crystalline form; but especially by its not fermenting with yeast and by yielding mucic acid with nitric acid.

For the preparation of milk-sugar we make use of the by-product in the preparation of cheese, the sweet whey. The proteid is removed by coagulation with heat and the filtrate evaporated to a syrup. The crystals which separate after a certain time are recrystallized from water after decolorizing with animal charcoal. A pure preparation may be obtained from the commercial milk-sugar by repeated recrystallization. The quantitative estimation of milk-sugar may in part be performed by the polaristrobometer and partly by means of titration with FEHLING'S solution. 10 c. c. of FEHLING'S solution corresponds to 0.067 grm. milk-sugar in 0.5–1.5% solution and boiling for 6 minutes (in regard to FEHLING'S solution and the titration of sugar, see Chapter XV).

RITTHAUSEN[1] has found another carbohydrate in milk which is soluble in water, non-crystallizable, which has a faint reducing action, and which yields on boiling with an acid a body having a greater reducing power. LANDWEHR[1] considers this as animal gum, and BÉCHAMP[2] as dextrin. According to J. HERZ[3] granules occur in milk, which act like starch with iodine and which are perhaps animal starch.

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[1] Journ. f. prakt. Chem., N. F., Bd. 15.

The *mineral bodies* of milk will be treated in connection with its quantitative composition.

The methods for the quantitative analysis of milk are very numerous, and as they cannot all be treated of here, we will give the chief points of a few of the most trustworthy and most frequently employed methods.

In determining the *solids* a carefully weighed quantity of milk is mixed with an equal weight of heated quartz sand, fine glass powder, or asbestos. The evaporation is first done on the water-bath and finished in a current of carbon dioxide or hydrogen not above 100° C.

The *mineral bodies* are determined by ashing the milk, using the precautions mentioned in the text-books. The results obtained for the phosphoric acid are incorrect on account of the burning of phosphorized bodies, such as casein and lecithin. We must therefore, according to SÖLDNER,[4] subtract 25% from the total phosphoric acid found in the milk. The quantity of sulphate in the ash also depends on the burning of the proteids.

In the determination of the *total amount of proteids* we make use of RITTHAUSEN'S[5] method, namely, precipitate the milk with copper sulphate. This method gives incorrect results because the copper hydroxide does not give up all its water of hydration on drying the precipitate, but only after ashing the same. The results for the proteids are therefore somewhat too high. I. MUNK[6] has modified this process in this wise, that he precipitates all the proteids by means of copper oxyhydrate at boiling heat and determines the nitrogen in the precipitate by means of KJELDAHL'S method. This modification gives exacter results.

The method of PULS[7] and STENBERG[8] consists in first diluting the neutralized milk with some water and then treating with alcohol until the mixture contains 70–85 vols. per cent alcohol. The precipitate is collected on a filter, washed with warm 70% alcohol, extracted with ether, dried, weighed, burnt, and the residue reweighed. The traces of proteid which remain in the filtrate and wash-liquor are precipitated by tannic acid. 63% of the tannic acid precipitate is considered as proteid, and this must be added to the proteid found directly. This method gives exact and good results, but is more complicated.

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[1] Pflüger's Arch., Bd. 39 and 40.

[2] Bull. soc. chim. (Ser. 3), Tome 6.

[3] Chem. Ztg.,Bd. 16, S. 1594.

[4] Landwirthsch. Versuchsstat., Bd. 35.

[5] Journal f. prakt. Chem., N. F., Bd. 15.

[6] Virchow's Arch., Bd. 134.

[7] Pflüger's Arch., Bd. 13.

[8] Nord. med. Arkiv., Bd. 9; also Maly's Jahresber., Bd. 7, S. 169.

According to SEBELIEN's[1] method, 3–5 grms. of milk are diluted with an equal volume of water, a little common-salt solution added, and precipitated with an excess of tannic acid. The precipitate is washed with cold water, and then the quantity of nitrogen determined by KJELDAHL's method. The total nitrogen found when multiplied by 6.37 (casein and lactalbumin contain both 15.7% nitrogen) gives the total quantity of albuminous bodies. This method, which is readily performed, gives very good results. I. MUNK used this method in the analysis of woman's milk. In this case the quantity of nitrogen found must be multiplied by 6.34. According to MUNK's analyses nearly $\frac{1}{16}$ of the total nitrogen of cow's milk and $\frac{1}{11}$ of woman's milk is derived from the extractives.

To determine the *casein* and *albumins* separately we may make use of the method first suggested by HOPPE-SEYLER and TOLMATSCHEFF,[2] in which the casein is precipitated by magnesium sulphate. According to SEBELIEN,[3] the milk is diluted with its own volume of a saturated magnesium-sulphate solution, then saturated with the salt in substance, the precipitate filtered and washed with a saturated magnesium-sulphate solution. The nitrogen is determined in the precipitate by KJELDAHL's method, and the quantity of casein determined by multiplying the result by 6.37. The quantity of lactalbumin may be calculated as the difference between the casein and the total proteids found. The lactalbumin may also be precipitated by tannic acid from the filtrate containing $MgSO_4$ from the casein precipitate, diluted with water, and the nitrogen determined by KJELDAHL's method and the result multiplied by 6.37.

The quantity of *globulins* in milk cannot be exactly determined. A minimum result can be obtained by first precipitating the casein completely by NaCl in substance, and then precipitating the globulins in the filtrate by magnesium sulphate (SEBELIEN). The casein may also be precipitated from the diluted milk by acetic acid and the globulin precipitated after neutralization by means of $MgSO_4$. In these cases we obtain somewhat high results, because of the presence of traces of casein which remain behind.

The *fat* is gravimetrically determined by thoroughly extracting the dried milk with ether, evaporating the ether from the extract, and weighing the residue. The fat may be determined by aerometric means by adding alkali to the milk, shaking with ether, and determining the specific gravity of the fat solution by means of SOXHLET's apparatus. In determining the amount of fat in a large number of samples the lactocrit of De Laval may be used with success. The milk is first mixed with an equal volume of a mixture of glacial acetic acid and concentrated sulphuric acid, warmed 7–8 minutes on the water-bath, the mixture placed in graduated tubes, and these in the centrifugal machine at + 50° C. The height of the layer of fat gives its quantity. The numerous and very exact analyses of Nilson[1] have shown that with milks containing small quantities of fat, below 1.5%, the older corrections are unnecessary, and that this method gives excellent results if we use lactic acid treated with 5% hydrochloric acid instead of the above mixture of glacial acetic acid and sulphuric acid.

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[1] Zeitschr. f. physiol. Chem., Bd. 13.

[2] Hoppe-Seyler, Med. chem. Untersuch., Heft 2.

[3] L. c.

In determining the *milk-sugar* first the proteids are removed. For this purpose we precipitate either with alcohol, which must be evaporated from the filtrate, or by diluting with water, and removing the casein by the addition of a little acid, and the lactalbumin by coagulation at boiling heat. The sugar is determined by titration with Fehling's or Knapp's solution (see Chap. XV). The principle of titration is the same as for the titration of sugar in urine: 10 c. c. of Fehling's solution corresponds to 0.0676 grm. milk-sugar; 10 c. c. of Knapp's solution corresponds to 0.0311–0.0310 grm. milk-sugar, when the saccharine liquid contains about ½–1% sugar. In regard to the *modus operandi* of the titration we must refer the reader to more complete works and to Chapter XV.

Instead of the volumetric determinations the following steps may be taken: A measured quantity of the milk-sugar solution is treated with an excess of Fehling's solution, boiled, the copper suboxide filtered and reduced in a current of hydrogen, and the metallic copper weighed. Soxhlet[2] has given a table which simplifies the calculations in such cases.

The sugar may also be determined by the polariscope, and with ease, because the filtrates containing milk-sugar are generally colorless. The determination is quickly performed, but does not give exact results.

The *quantitative composition* of cow's milk is naturally ve variable. The average obtained by König[3] is as follows in 10 parts:

Water.	Solids.	Casein.	Albumin.	Fats.	Sugar.	Sal
871.7	128.3	30.2	5.3	36.9	48.8	7.1
		35.5				

The quantity of *mineral bodies* in 1000 parts of cow's milk i according to the analyses of Söldner,[4] as follows: K_2O 1.72, Na_2

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[1] Maly's Jahresber., Bd. 21, S. 142.

[2] Journal f. prakt. Chem., 1880.

[3] Chemie der menschichen Nährungs- und Genussmittel, 3. Aufl.

[4] L. c.

0.51, CaO 1.98, MgO 0.20, P_2O_5 1.82 (after correction for the pseudonuclein), Cl 0.98 grms. **Bunge**[1] found 0.0035 grm. Fe_2O_3. According to **Söldner**, the K, Na, and Cl are found in the same quantities in whole milk as in milk-serum. Of the total phosphoric acid 36–56% is not simply dissolved and also 53–72% of the lime. A part of this lime is combined with the casein; the remainder is found united with the phosphoric acid as a mixture of di- and tri-calcium pohsphate, which is kept dissolved or suspended by the casein. The bases are in excess of the mineral acids in the milk-serum. The excess of the first is combined with organic acids, which correspond to 2.5 p. m. citric acid (**Söldner**[2]).

The *gases* of the milk consist chiefly of CO_2, besides a little N and traces of O. **Pflüger**[3] found 10 vols. per cent CO_2 and 0.6 vol. per cent N, calculated at 0° C. and 760 mm. pressure.

The variation in the composition of cow's milk depends on several circumstances.

The **colostrum**, or the milk which is secreted before calving and in the first few days after, is yellowish, sometimes alkaline, but often acid, of higher specific gravity, 1.046–1.080, and richer in solids than ordinary milk. The colostrum contains, besides fat-globules, an abundance of colostrum-corpuscles—nucleated granular cells 0.005–0.025 mm. in diameter with abundant fat-granules and fat-globules. The fat of colostrum has a somewhat higher melting-point and is poorer in volatile fatty acids than the fat from ordinary milk (**Nilson**[4]). The quantity of cholesterin and lecithin is generally greater. The most apparent difference between it and ordinary milk is that colostrum coagulates on heating to boiling because of the absolute and relatively greater quantities of globulin and albumin it contains. The quantity of the first of these two albuminous bodies may indeed amount to several per cent (**Sebelien**[5]). The composition of colostrum is very variable. **König**[6] gives as average the following figures in 1000 parts:

Water.	Solids.	Casein.	Albumin and Globulin.	Fat.	Sugar.	Salts.
746.7	253.3	40.4	136.0	35.9	26.7	15.6

The constitution of milk is changed during lactation, and it becomes richer in casein but poorer in fat and milk-sugar. The evening milk is richer in fat than the morning milk (ALEX. MÜLLER and EISENSTUCK; NILSON and others[1]). The breed of the animal also has a great influence on the milk.

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[1] Zeitschr. f. Biologie, Bd. 10.

[2] L. c.

[3] Pflüger's Arch., Bd. 2.

[4] Maly's Jahresber., Bd. 17, S. 169.

[5] *Ibid.*, Bd. 18, S. 102.

[6] L. c.

The influence food exercises upon the composition of milk will be discussed in connection with the chemistry of the milk secretion.

In the following we give the average composition of skimmed milk and certain other preparations of milk:

	Water.	Proteids.	Fat.	Sugar.	Lactic Acid.	Salts.
Skimmed milk.	906.6	31.1	7.4	47.5	....	7.4
Cream.........	655.1	36.1	267.5	35.2	....	6.1
Buttermilk....	902.7	40.6	9.3	37.3	3.4	6.7
Whey	932.4	8.5	2.3	47.0	3.3	6.5

KUMYSS and KEPHIR are obtained, as above stated, by the alcoholic and lactic-acid fermentation of the milk-sugar, the first from mare's milk and the last from cow's milk. Large quantities of carbon dioxide are formed thereby, and also the albuminous bodies of the milk are partly converted into albumoses and peptones, which increases the digestibility. The quantity of lactic acid in these preparations may be about 10–20 p. m. The quantity of alcohol varies from 10 to 35 p. m.

Milk from other Animals. GOAT'S milk has a more yellowish color and another, more specific, odor than cow's milk. The coagulation obtained by acid or rennet is more solid and is harder than that from cow's milk. SHEEP'S milk is similar to goat's milk, but has a higher specific gravity and contains a greater amount of solids.

MARE'S milk is alkaline and contains a casein which is not precipitated by acids in lumps or solid masses, but, like the casein from woman's milk, in fine flakes. This casein is only incompletely precipitated by rennet, and it is very similar also in other respects to the casein of human milk. According to BEIL,[2] the casein from mare's and cow's milk is the same, and the different behavior of the two varieties of milk is due to different amounts of salts and to a different relation between the casein and the albumin. The milk of the ASS is similar to human milk.

The milk of CARNIVORA, the bitch and cat, are acid in reaction and very rich in solids. The composition of the milk of these animals varies very much with the composition of the food.

To illustrate the composition of the milk of other animals the following figures, the compilation of KÖNIG, will be given. As the milk of each variety of animals may have a variable composition, these figures may only be considered as examples of the composition of milk of different kinds.

Milk of the	Water.	Solids.	Proteids.	Fat.	Sugar.	Salts.
Dog	754.4	245.6	99.1	95.7	31.9	7.3
Cat.........	816.3	183.7	90.8	33.3	49.1	5.8
Goat	869.1	130.9	36.9	40.9	44.5	8.6
Sheep......	835.0	165.0	57.4	61.4	39.6	6.6
Cow	871.7	128.3	35.5	36.9	48.8	7.1
Horse......	900.6	99.4	18.9	10.9	66.5	3.1
Ass	900.0	100.0	21.0	13.0	63.0	3.0
Pig	823.7	167.3	60.9	64.4	40.4	10.6
Elephant..	678.5	321.5	30.9	195.7	88.4	6.5
Dolphin[3]..	468.7	513.3	....	437.5	....	4.6

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[1] See König, l. c., Bd. 1, S. 313, and Nilson, l. c.

[2] Studien über die Eiweissstoffe des Kumys und Kefir. St. Petersb 1886. (Ricker.)

[3] Frankland, Chem. News, 1890, vol. 61.

Human Milk.

Woman's milk is amphoteric in reaction. According to COURANT[1] its reaction is relatively stronger alkaline than cow's milk, but has nevertheless a lower absolute reaction for alkalinity as well as acidity. COURANT found between the tenth day and fourteenth month after confinement rather constant results. The alkalinity as well as the acidity were a little lower than in childbed. 100 c. c. of the milk had the same average alkalinity as 10.8 c. c. $\frac{n}{10}$ caustic soda and the same acidity as 3.6 c. c. $\frac{n}{10}$ acid. The relationship between the alkalinity and acidity was for woman's milk as 3 : 1, and in cow's milk as 2.1 : 1.

Human milk also contains fewer fat-globules than cow's milk, but they are larger in size. The specific gravity of woman's milk varies between 1026 and 1036, generally between 1028 and 1034. According to MONTI[2] the specific gravity of the milk from healthy, robust women is 1030–1035. The specific gravity is highest in well-fed and lowest in poorly fed women.

The fat of woman's milk has been investigated by RUPPEL.[3] It forms a yellowish, white mass, similar to ordinary butter, having a specific gravity of 0.966 at + 15° C. It melts at 34.0° and solidifies at 20.2° C. The following fatty acids can be obtained from the fat, namely, butyric, caproic, capric, myristic, palmitic, stearic, and oleic acids. The fat from woman's milk is, according to RUPPEL, relatively poor in volatile fatty acids. LAVES[4] found only traces of butyric acid in the fat from woman's milk. The melting-point of the fat was 30–31° and of the free fatty acids 37–39° C. The non-volatile fatty acids consist of one-half oleic acid, while among the solid fatty acids myristic and palmitic acids are found to a greater extent than stearic acid.

The essential qualitative difference between woman's and cow's milk seems to lie in the proteids or in the more accurately determined *casein*. A number of older and younger investigators[5] claim that the casein from woman's milk has other properties than that from cow's milk. The essential differences are the following: The casein from woman's milk is precipitated with greater difficulty with acids or salts; it does not coagulate regularly in the milk after the addition of rennet; it may be precipitated by gastric juice, but dissolves completely and easily in an excess of the same; the casein precipitate produced by an acid is more easily soluble in an excess of the acid; and lastly, the clot formed from the casein of woman's milk does not appear in such large and coarse masses as the casein from cow's milk, but is more loose and flocculent. This last-mentioned fact is of great importance, since it explains the generally admitted easy digestibility of the casein from woman's milk. The question as to whether the above-mentioned differences depend on a decided difference in the two caseins or only on an unequal relationship between the casein and the salts in the two varieties of milk, or upon other circumstances, has been recently investigated. According to SZONTAGH[1] the casein from human milk does not yield any pseudonuclein on pepsin digestion and hence it cannot be a nucleoalbumin. WRÓBLEWSKI[2] has recently arrived at the same results and also found that the two caseins had a different composition. He found the following for the composition of casein from woman's milk: C 52.24, H 7.32, N 14.97, P 0.68, S 1.117, O 23.66%. Woman's milk also contains lactalbumin besides the casein and a protein substance which is very rich in sulphur (4.7%) and relatively poor in carbon (WRÓBLEWSKI). The statements as to the occurrence of albumoses and peptones are disputed as in many other cases. No positive proof as to the occurrence of albumoses and peptones in fresh milk has been given.

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[1] Ueber die Reaktion der Kuh- und Frauenmilch, etc. Inaug. Diss. Bonn, 1891; also Pflüger's Arch., Bd. 50.

[2] Arch. f. Kinderheilkunde, Bd. 13.

[3] Zeitsch. f. Biologie, Bd. 31.

[4] Zeitschr. f. physiol. Chem., Bd. 19.

[5] See Biedert, Untersuchungen über die chemischen Unterschiede der

The quantitative composition of woman's milk is, even after those differences are eliminated which depend on the imperfect analytical methods employed, variable to such an extent that it is impossible to give any average results. Eliminating certain of the older, incorrect analyses, we here give only examples from the average results of a few modern investigators, taken from a very large number of analyses (PFEIFFER). The following figures are parts per 1000:

Menschen- und Kuhmilch. Stuttgart, 1884. Langgaard, Virchow's Arch., 65. Makris, Studien über die Eiweisskörper der Frauen- und Kuhmil Inaug. Diss. Strassburg, 1876.

[1] Maly's Jahresber., Bd. 22, S. 168.

ge zur Kenntniss des Frauenkaseïns. Inaug.-Diss. Bern, 1894.

Water.	Solids.	Proteids.	Fat.	Cholesterin.	Sugar.	Salts.		
876.0	124.0	22.10	38.10	......	60.90	2.90	Biel[1]	
......	......	23 60	25.60	0.32	55.60	......	Tolmatscheff[2]	
891.0	109.0	17.90	33.00	......	53.90	4.20	Gerber[3]	
872.4	127.6	19 00	43.20	......	59.70	2.80	Christenn[4]	
892.0	108.0	16.13	32.28	......	57.94	1.65	20–30yrs.old	Pfeiffer[5]
890.6	109.4	17.24	29.15	......	59.92	2.09	30–40 " "	
877.9	122.1	25.30	38.90	. ....	55.40	2.50	Mendes de Leon[6]	

Although the composition of woman's milk is very variable, and notwithstanding that in a few cases higher results (about 40 p. m.) have been obtained, by later analyses, for proteid bodies, still it seems that woman's milk in general contains less proteids and more sugar than cow's milk. The quantity of casein is not only absolutely but also relatively smaller in proportion to the quantity of albumin in woman's than in cow's milk. According to Scheibe[7] the quantity of citric acid is smaller in woman's milk than in cow's milk.

A further difference between woman's and cow's milk is that the first is richer in lecithin but poorer in mineral bodies, especially CaO and P_2O_5 (it contains only $\frac{1}{6}$ and $\frac{1}{4}$, respectively, of the corresponding quantity of these mineral bodies in cow's milk).

In regard to the quantity of *mineral bodies* in woman's milk the analyses of Bunge[8] are most reliable. He analyzed the milk of a woman, fourteen days after delivery, whose diet contained very little common salt for four days previous to the analysis (A), and again three days later after a daily addition of 30 grms. NaCl to the food (B). Bunge found the following figures in 1000 parts of the milk:

	A	B
K_2O	0.780	0.703
Na_2O	0.232	0.257
CaO	0.328	0 343
MgO	0.064	0.065
Fe_2O_3	0.004	0.006
P_2O_5	0.473	0.469
Cl	0.438	0.445

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[1] Maly's Jahresber., Bd. 4, S. 168.

[2] Hoppe-Seyler, Med. chem. Untersuch., Heft 2.

[3] Bull. de la soc. chim., Tome 23.

[4] Maly's Jahresber., Bd. 7, S. 171.

[5] Jahrb. f. Kinderheilkunde, Bd. 20; also Maly's Jahresber., Bd. 13.

[6] Ueber die Zusammensetzung der Frauenmilch. Inaug. Diss. der Univ. Heidelberg. 1881; also Maly's Jahresber., Bd. 12.

[7] Landwirthsch. Versuchsstat., Bd. 39.

[8] Zeitschr. f. Biologie, Bd. 10.

The relationship of the two bodies, potassium and sodium, to each other may, according to BUNGE, vary considerably (1.3–4.4 equivalents potash to 1 of soda). By the addition of salt to the food the quantity of sodium and chlorine in the milk increases, while the quantity of potassium decreases. The gases of woman's milk have been investigated by KULZ.[1] He found 1.07–1.44 c. c. oxygen, 2.35–2.87 c. c. carbon dioxide, and 3.37–3.81 c. c. nitrogen in 100 c. c. milk.

The proper treatment of cow's milk by diluting with water and by certain additions in order to render it a proper substitute for woman's milk in the nourishment of babes cannot be determined before the difference in the albuminous bodies of these two kinds of milk has been completely studied.

The **colostrum** has a higher specific gravity, 1.040–1.060, a greater quantity of coagulable proteids, and a deeper yellow color than ordinary woman's milk. Even a few days after delivery the color becomes less yellow, the quantity of albumin less, and the number of colostrum-corpuscles diminishes. CLEMM[2] has analyzed the colostrum at different periods before and after delivery, and the following are his results in parts per 1000:

	Four Weeks before Delivery.		Seventeen Days before Delivery.	Nine Days before Delivery.	Twenty-four Hours after Delivery.	Two Days after Delivery.
	1	2				
Water	945.2	852.0	851.7	858.5	843.0	867.9
Solids	54.8	148.0	148.3	141.5	157.0	132.1
Casein	......	....	....	....	....	21.8
Albumin	28 8	69.0	74.8	80.7	....	....
Fat	7.1	41.3	30.2	23.5	....	48.6
Milk-sugar	17.3	39.5	43.7	36.4	....	61.0
Salts	4.4	4 4	4.5	5.4	5.1	....

The total quantity of proteids seems to decrease with the du[illegible]ration of lactation. PFEIFFER[3] found the average figures for [illegible]he total proteids for the two first days, the first week, the second we[illegible]k the second month, and the seventh month to be 86.04, 34.[illegible] 22.88, 18.43, and 15.21 p. m., respectively. SIMON[4] claims t[illegible] the amount of casein is smaller in the first stages of lactation a[illegible] then increases considerably; but according to Pfeiffer just the reverse takes place. The amount of fat shows no regular and constant variation during lactation. According to Vernois and Becquerel[1] the quantity of milk-sugar decreases in the first months, but increases in the eighth to the tenth month. According to Pfeiffer the quantity of sugar increases regularly from the delivery to the third to fourth month, and then it is somewhat variable.

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[1] Zeitschr. f. Biologie, Bd. 32.

[2] Cited from Hoppe-Seyler's Physiol. Chem., p. 784.

[3] L. c.

[4] Die Frauenmilch. Berlin, 1888.

The two mammary glands of the same woman may yield somewhat different milk, as shown by Sourdat[2] and later by Brunner.[3] Also the different portions of milk from the same milking may have different compositions. The first portions are always poorer in fat.

According to L'Héritier,[4] Vernois, and Becquerel the milk of blonds contains less casein than that of brunettes, a difference which Tolmatscheff[5] could not substantiate. Women of weak constitutions yield a milk richer in solids, especially in casein, than women with strong constitutions (V. and B.).

According to Vernois and Becquerel, the age of the woman has an effect on the composition of the milk, so that we find a greater quantity of proteids and fat in women 15–20 years old and a smaller quantity of sugar. The smallest quantity of proteids and the greatest quantity of sugar are found at 20 or from 25-30 years of age. According to V. and B., the milk with the first-born is richer in water—with a proportionate diminution of the quantity of casein, sugar, and fat—than after several deliveries.

The influence of menstruation seems to slightly diminish the milk-sugar and to considerably increase the fat and casein (V. and B.).

Witch's Milk is the secretion of the mammary glands of new-born children of both sexes immediately after birth. This secretion has from a qualitative standpoint the same constitution as milk, but may show important differences and variations from a quantitative point of view. Schlossberger and Hauff,[6] Gubler and Quevenne,[7] and v. Genser[8] have made analyses of this milk and give the following results: 10.5–28 p. m. proteids, 8.2–14.6 p. m. fat, and 9–60 p. m. sugar.

As milk is the only form of nourishment during a certain period of the life of man and mammals, it must contain all the nutritious bodies necessary for life. This fact is shown by the milk-containing representatives of the three chief groups of organic nutritive substances, proteids, carbohydrates, and fat; and all milk seems to contain also some lecithin. The mineral bodies in milk must also occur in proper proportion, and on this point the observations of

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[1] Compt. rend., Tome 36, and Vernois et Becquerel, Du lait chez la femme dans l'état de santé, etc. Paris, 1853.

[2] Compt. rend., Tome 71.

[3] Pflüger's Arch., Bd. 7.

[4] Traité de chim. pathol. Paris, 1842. Cited from Hoppe-Seyler's Physiol. Chem., p. 738.

[5] Hoppe-Seyler, Med. chem. Untersuch., S. 272.

[6] Annal. de Chem. u. Pharm., Bd 96.

[7] Gaz. méd. de Paris, 1856 p. 15.

[8] Jahrb. f. Kinderheilkunde, N. F., Bd. 9, S. 60.

Bunge on dogs are of special interest. He found that the mineral bodies of the milk occur in about the same relative proportion as they do in the body of the sucking animal. **Bunge**[1] found in 1000 parts of the ash the following results (A represents results from the new-born dog and B the milk from the bitch):

	A	B
K_2O	114.2	149.8
Na_2O	106.4	88.0
CaO	295.2	272.4
MgO	18.2	15.4
Fe_2O_3	7.2	1.2
P_2O_5	394.2	342.2
Cl	83.5	169.0

Bunge explains the fact that the milk-ash is richer in potash and poorer in soda than the new-born animal by saying that in the growing animal the ash of the muscles rich in potash relatively increases and the cartilage rich in soda relatively decreases. **Bunge** seeks to explain the high amount of chlorine in the milk-ash also teleologically by the statement that the chlorides not only serve to build up the tissues, but are indispensable in the secretions of the kidneys. In regard to the amount of iron we find an unexpected condition, the ash of the new-born animal containing six times as much as the milk-ash. This condition **Bunge** explains by the fact founded on his and **Zalesky**'s experiments, that the quantity of iron in the total organism is highest at birth. The new-born animal has therefore a supply of iron for the growth of its organs even at its birth.

The *influence of the food* on the composition of the milk is of interest from many points of view and has been the subject of many investigations. From these investigations we learn that in human beings as well as in animals an insufficient diet decreases the quantity of milk and the quantity of solids in the same, while abundant food increases both. From the observations of **Decaisne**[2] on nursing women during the siege of Paris in 1871, the quantity of casein, fat, sugar, and salts, but especially the fat, was found to decrease with insufficient food, while the quantity of lactalbumin was found to be somewhat increased. Food rich in proteids increases the quantity of milk, and also the solids contained, especially the fat. The quantity of sugar in woman's milk is found by certain investigators to be increased after food rich in proteids, while others claim it is diminished. Food rich in fat may (in sheep) cause an increase in the quantity of fat in the milk. An increase in the quantity of fat in cow's milk because of an addition of fat to the fodder has only been observed after a previous insufficient diet, but not after a sufficient and rich diet. After feeding with palm-oil cake a one-sided increase in the fat of cow's milk was observed. The presence of large quantities of carbohydrates in the food seems to cause no constant, direct action on the quantity of the milk-constituents.[1] In carnivora, as shown by SSUBOTIN,[2] the secretion of milk-sugar proceeds uninterruptedly on a diet consisting exclusively of lean meat. Watery food gives a milk containing an excess of water of little value. In the milk from cows which were fed on distillers' grains COMMAILLE[3] found 906.5 p. m. water, 26.4 p. m. casein, 4.3 p. m. albumin, 18.2 p. m. fat, and 33.8 p. m. sugar. Such milk has a peculiar sour, sharp after-taste.

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[1] Zeitschr. f. physiol. Chem., Bd. 13.

[2] Gaz. méd. de Paris, 1871, S. 317; cited from Hoppe-Seyler, l. c., S. 739.

Chemistry of the Milk-secretion. That the actually dissolved constituents occurring in milk pass into the secretion, not alone by filtration or diffusion, but more likely are secreted by a specific secretory activity of the glandular elements, is shown by the fact that milk-sugar, which is not found in the blood, is to all appearances formed in the glands themselves. A further proof lies in the fact that the lactalbumin is not identical with seralbumin; and lastly, as BUNGE[4] has shown, the mineral bodies secreted by the milk are in quite different proportions from those in the blood-serum.

Little is known in regard to the formation and secretion of the specific constituents of milk. The older theory, that the casein was produced from the lactalbumin by the action of an enzyme, is incorrect and originated probably from mistaking an alkali-albuminate for casein. Better founded is the statement that the casein originates from the protoplasm of the gland-cells, which seem to consist of casein or a substance related to it. The previously mentioned (page 420) nucleoproteid of the gland-cells appears to be related to casein, and it may possibly form its mother-substance. There does not seem to be any doubt that the protoplasm of the cells takes part in the secretion in such a manner that it becomes itself a constituent of the secretion. According to HEIDENHAIN,[1] the alveoles contain a simple layer of cells, which, in the inactive gland, are flat, polyhedrous, and with single nucleus, while in the active gland they often have several nuclei, are rich in proteid, and are high and cylindrical in form. In the inner part of the cell turned towards the cavity of the acinus, single fat-granules are formed during the secretion which are broken off with the edge of the cells. The broken-off or destroyed cell-substance in the secretion dissolves in the milk, filling the lumen of the acinus, while the cells take up nutrition by their outer parts, and grow, and replace the inner parts used in the secretion. This reminds us of the action of the pancreas-cells in the secretion of the pancreatic juice. The colostrum-corpuscles are not, according to HEIDENHAIN, degenerated fat-cells, but are contractile elements originating from the epithelium, which take up finely divided fat and thereby obtain their quantity of fat-globules.

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[1] In regard to the literature on the action of various foods on woman's milk, see Zalesky, "Ueber die Einwirkung der Nahrung auf die Zusammensetzung und Nahrhaftigkeit der Frauenmilch," Berlin. klin. Wochenschr., 1888, which also contains the literature on the importance of the food on the composition of other varieties of milk. In regard to the extensive literature on the influence of various foods on the milk production of animals, see König, Chem. d. menschl. Nahrungs- und Genussmittel, 3. Aufl., Bd. 1, S. 298.

[2] Centralbl. f. d. med. Wissensch., 1866, S. 337.

[3] Cited from König, Bd. 2, S. 235.

[4] Lehrbuch d. physiol. und pathol. Chem., 1. Aufl., S. 98.

That the milk-fat is produced by a formation of fat in the protoplasm, and that the fat-globules are set free by their destruction, is a generally admitted opinion which, however, does not exclude the possibility that the fat is in part taken up by the glands from the blood and eliminated with its secretion. A formation of fat from carbohydrates in the animal organism is at the present day considered as positively proved, and it is also possible that the milk-glands also produce fats from the carbohydrates brought to them by the blood. It is a well-known fact that an animal gives off for a long time, daily, considerably more fat in the milk than it receives as food, and this proves that at least a part of the fat secreted by the milk is produced from proteids or carbohydrates, or perhaps from both. The question as to how far this fat is produced directly in the milk-glands, or from other organs and tissues, and brought to the gland by means of the blood, cannot be decided.

The origin of the milk-sugar is not known. MÜNTZ[2] calls attention to the fact that a number of very widely diffused bodies in the vegetable kingdom—vegetable mucilage, gums, pectin bodies—yield galactose as products of decomposition, and he believes, therefore, that the milk-sugar may be formed in herbivora by a synthesis from dextrose and galactose. This origin of milk-sugar does not answer for carnivora, as they produce milk-sugar when fed on food consisting entirely of lean meat. The observations of Bert and Thierfelder[1] that a mother-substance of the milk-sugar, a *saccharogen*, occurs in the glands cannot, as the nature of this mother-substance is still unknown, give further explanation as to the formation of milk-sugar. The question whether the above-mentioned (page 420) proteid, which yields a reducing substance when boiled with dilute acids, has anything to do with the formation of milk-sugar cannot be answered until further thorough investigations have been made on this subject.

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[1] Hermann's Handbuch d. Physiol., Bd. 5, Thl. 1, S. 380.

[2] Compt. rend., Tome 102.

The passage of foreign substances into the milk stands in close connection with the chemical processes of the milk-secretion.

It is a well-known fact that milk acquires a foreign taste from the food of the animal, which is in itself a proof that foreign bodies pass into the milk. This fact becomes of special importance in reference to such injurious substances as may be introduced into the organism of the nursing child by means of the milk.

Among these substances may be mentioned opium and morphine, which after large doses pass into the milk and act on the child. Alcohol may also pass into the milk, but not probably in such quantities as to have any direct action on the nursing child.[2] Alcohol is claimed to have been detected in the milk after feeding cows with brewer's grains.

Among inorganic bodies, iodine, arsenic, bismuth, antimony, zinc, lead, mercury, and iron have been found in milk. In icterus neither bile-acids nor bile-pigments pass into the milk.

Under diseased conditions no constant change has been found in woman's milk. In isolated cases Schlossberger,[3] Joly and Filhol[4] have observed indeed a markedly abnormal composition, but no positive conclusion can be derived therefrom.

The changes in cow's milk in disease have been little studied. In tuberculosis of the udder Storch[5] found tubercule bacilli in the milk, and he also found that the milk became more and more diluted during the disease with a serous liquid similar to blood-serum, so that the glands finally, instead of yielding milk, only gave blood-serum or a serous fluid. HUSSON[1] found the milk from cows sick with murrain contained more proteids but considerably less fat and (in difficult cases) less sugar than normal milk.

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[1] L. c.

[2] Klingemann, Virchow's Arch., Bd. 126.

[3] Annal. d. Chem. u. Pharm., Bd. 96.

[4] Cited from v. Gorup-Besanez, Lehrb., 4. Aufl., S. 438.

[5] See Bang, Om Tuberkulose i Koens Yver og om tuberkulös Mälk. Nord. med. Arkiv., Bd. 16; also Maly's Jahresber., Bd. 14, S. 170.

The milk may be blue or red in color, due to the development of micro-organisms.

The formation of concrements in the exit-passages of the cow's udder are often observed. They consist chiefly of calcium carbonate, or of carbonate and phosphate with only a small amount of organic substances.

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[1] Compt. rend., Tome 73.

CHAPTER XV.

THE URINE.

THE urine is the most important excretion of the animal organism; it is the means of eliminating the nitrogenous metabolic products, also the water and the soluble mineral substances; and in many cases it furnishes important data relative to the metabolism, quantitatively by its variation, and qualitatively by the appearance of foreign bodies in the excretion. Also in many cases we are able from the chemical or morphological constituents which the urine abstracts from the kidneys, ureters, bladder, and urethra to judge of the condition of these organs; and lastly, urinary analysis affords an excellent means of deciding the question how certain medicines or other foreign substances introduced into the organism are absorbed and chemically changed. Urinary analysis has furnished very important particulars especially relative to the last-mentioned question in regard to the nature of the chemical processes taking place within the organism, and it is therefore not only an important aid in diagnosis to the physician, but it is also of the greatest importance to the toxicologist and the physiological chemist.

In studying the secretions and excretions the relationship must be sought between the chemical structure of the secreting organ and the chemical composition of its secreted products. Investigations with respect to the kidneys and the urine have led to very few results from this standpoint. Although the anatomical relation of the kidneys has been carefully studied, their chemical composition has not been the subject of thorough analytical research. In cases in which a chemical investigation of the kidneys has been undertaken, it has only been in general on the organ as such, and not on the different anatomical parts. An enumeration of the chemical constituents of the kidneys known at the present time can, therefore, only have a secondary value.

In the kidneys we find albuminous bodies of different kinds. According to HALLIBURTON[1] the kidneys do not contain any albumin, but only *globulin* and *nucleoalbumin*. The globulin coagulates at about 52° C. and the nucleoalbumin at 63° C. The quantity of phosphorus in the latter is 0.37%. According to LIEBERMANN[2] the kidneys contain *lecithalbumin*, and he ascribes to this body a special importance in the secretion of acid urines, namely, he claims that the lecithalbumin, which acts like an acid, decomposes in part the alkali-salts of the blood-plasma in the cells, combining with the alkalies. Besides the above protein substances and the albuminoids of the connective-substance group, the kidneys contain a *body similar to mucin*. The question as to whether pure mucin really exists in the kidneys has not been decided. The body similar to mucin, which is a nucleoalbumin, and which gives no reducing substance when boiled with acids (LÖNNBERG[3]), belongs chiefly to the papillæ, while the cortical substance is richer in a non-mucin-like nucleoalbumin.

Fat occurs only in very small amounts in the cells of the tortuous urinary passages. Among the extractive bodies of the kidneys we find *xanthin bodies*, also *urea*, *uric acid* (traces), *glycogen*, *leucin*, *inosit*, *taurin*, and *cystin* (in ox-kidneys). The quantitative analyses of the kidneys thus far made possess little interest. OIDTMANN[4] found 810.94 p. m. water, 179.16 p. m. organic and 0.99 p. m. inorganic substance in the kidney of an old woman.

The fluid collected under pathological conditions, as in hydronephrosis, is thin with a variable but generally low specific gravity. Usually it is straw-yellow or paler in color, and sometimes colorless. Most frequently it is clear, or only faintly cloudy from white blood-corpuscles and epithelium-cells; in a few cases it is so rich in form-elements that it appears like pus. Proteids occur generally only in small amounts; sometimes it is entirely absent, and in a few rare cases the amount is nearly as large as in the blood-serum. Urea occurs sometimes in considerable amounts when the parenchyma of the kidneys is only in part atrophied; in complete atrophy the urea may be entirely absent.

I. Physical Properties of Urine.

Consistency, transparency, odor, and taste of urine. Urine is under physiological conditions a thin liquid and gives, when shaken with air, a froth which quickly subsides. Human urine or urine from carnivora, which is habitually acid, appears clear and transparent, often faintly fluorescent, immediately after voiding. When allowed to stand for a little while human urine shows a light cloud (*nubecula*) which consists of the so-called "mucus" and generally also contains a few epithelium-cells, mucus-corpuscles, and urate-granules. The presence of a larger quantity of urates renders the urine cloudy, and a clay-yellow, yellowish-brown, rose-colored, or often brick-red precipitate (*sedimentum lateritium*) settles on cooling because of the greater insolubility of the urates at the ordinary temperature than at the temperature of the body. This cloudiness disappears on gently warming. In new-born infants the cloudiness of the urine during the first 4–5 days is due to epithelium, mucus-corpuscles, uric acid, and urates. The urine of herbivora, which is habitually neutral or alkaline in reaction, is very cloudy on account of the carbonates of the alkaline earths present. Human urine may sometimes be alkaline under physiological conditions. In this case it is made cloudy by the earthy phosphates, and this cloudiness does not disappear on warming, differing in this respect from the *sedimentum lateritium*. Urine has a salty and faintly bitter taste produced by sodium chloride and urea. The odor of urine is peculiarly aromatic; the bodies which produce this odor are unknown.

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[1] Journal of Physiol., Vol. 13, Suppl.

[2] Pflüger's Arch., Bdd. 50 and 54.

[3] See Maly's Jahresber., Bd. 20.

[4] Cited from v. Gorup-Besanez, Lehrb., 4. Aufl., S. 732.

The **color** of urine is normally pale yellow when the specific gravity is 1.020. The color otherwise depends on the concentration of the urine and varies from pale straw-yellow, when the urine contains small amounts of solids, to a dark reddish yellow or reddish brown in stronger concentration. As a rule the intensity of the color corresponds to the concentration, but under pathological conditions exceptions occur, and such an exception is found in diabetic urine, which contains a large amount of solids and has a high specific gravity and a pale yellow color.

The **reaction** of urine depends essentially upon the composition of the food. The carnivora void an acid, the herbivora a neutral or alkaline, urine. If a carnivora is put on a vegetable diet, its urine may become less acid or neutral, while the reverse occurs when an herbivora is starved, that is, when it lives upon its own flesh, as then the urine voided is acid.

The urine of a healthy man on a mixed diet has an *acid reaction*, and the sum of the acid equivalents is greater than the sum of the base equivalents. This depends on the fact that in the physiological combustion of neutral substances (proteids and others) within the organism acids are produced, chiefly sulphuric acid, but also phosphoric and organic acids, such as hippuric, uric, and oxalic acid, also aromatic oxyacids and others. From this it follows that the acid reaction is not due to one acid alone. We do not know to what extent any one acid takes part in the acid reaction; but as the sum of the base equivalents is greater than, or at least the same as, the sum of the inorganic acid equivalents, the acid reaction must be due in greatest part to organic acids or acid salts. It is generally considered that the acid reaction of human urine is caused by double-acid alkali-phosphate (monophosphate). The quantity of acid-reacting bodies or combinations eliminated by the urine in 24 hours, when calculated as oxalic acid or hydrochloric acid, is respectively 2–4 and 1.15–2.3 grms.

The composition of the food is not the only influence which affects the degree of acidity of human urine. For example, after taking food, at the beginning of digestion, when a larger amount of gastric juice containing hydrochloric acid is secreted, the urine may be neutral or even alkaline. The statements of various investigators are rather contradictory in regard to the time of the appearance of the maximum and minimum of the acidity, which may in part be explained by the different individuality and different conditions of life of the persons investigated. It has not infrequently been observed that perfectly healthy persons in the morning void a neutral or alkaline urine which is cloudy from earthy phosphates. The effect of muscular activity on the acidity of urine has not been positively determined. According to HOFFMANN[1] and RINGSTEDT[2] muscular work raises the degree of acidity, but ADUCCO[3] claims that it decreases it. Abundant perspiration reduces the acidity (HOFFMANN).

In man and carnivora it seems that the degree of acidity of the urine cannot be increased above a certain point, even though mineral acids or organic acids which are burnt up with difficulty are taken in large quantities. When the supply of carbonates of the fixed alkalies stored up in the organism for this purpose is not sufficient to combine with the excess of acid, then ammonia is split from the proteids or their decomposition products, and the excess of acid combines therewith, forming ammonium salts which pass into the urine. In herbivora this splitting of ammonia and formation of ammonia salts does not seem to take place, and the herbivora therefore soon die when acids are given. Nevertheless the degree of acidity of human urine may be easily diminished so that the reaction is neutral or alkaline. This occurs after the taking of carbonates of the fixed alkalies or of such salts of vegetable acids—tartaric-acid, citric-acid, and malic-acid salts—as are easily burnt into carbonates in the organism. Under pathological conditions, as in the absorption of alkaline transudations, the urine may become alkaline (QUINCKE[1]).

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[1] Zur Semiologie des Harns. Inaug.-Diss. Berlin, 1884. See Maly's Jahresber., Bd. 14, S 213.

[2] See Maly's Jahresber., Bd. 20, S. 196.

[3] *Ibid.*, Bd. 17, S. 179.

The *degree of acidity* cannot be *determined* by the ordinary acidimetric process, since the urine contains di-hydrogen phosphate, MH_2PO_4, besides hydrogen di-phosphate, M_2HPO_4. In the titration the di-hydrogen phosphate is changed gradually into M_2HPO_4, and we obtain at a certain point a mixture of the two phosphates in variable proportions, which mixture is not neutral but amphoteric. Since it is generally admitted that the acid reaction of urine is due to the di-hydrogen phosphate, it is therefore best to express the degree of acidity by the amount of di-hydrogen phosphate present.

If we wish to calculate the degree of acidity of the urine as di-hydrogen phosphate or, still more simply, as phosphoric anhydride, P_2O_5, contained in this salt, the titration is performed according to the method of MALY and HOFFMANN,[2] which is as follows: The urine (100–200 c. c.) is treated with an exactly measured quantity of $\frac{1}{4}$ normal caustic-soda solution, which is more than sufficient to convert all the phosphate into basic phosphate, or, in other words, enough to make the urine strongly alkaline. Then an approximate $\frac{2}{3}$ normal $BaCl_2$ solution (142.8 grms. $BaCl_2,2H_2O$ in a litre) is added until no further precipitate is formed. By this means all the phosphoric acid is precipitated from the urine. Filter through a dry filter, measure a quantity corresponding to 50 or 100 c. c. of the original urine from the filtrate, and titrate with $\frac{1}{4}$ normal sulphuric acid until a neutral reaction is obtained, using litmus-paper as an indicator. If the amount found by this titration be subtracted from the original amount of caustic soda added to this volume of urine, the difference is the amount of caustic soda necessary to convert the existing di-hydrogen and hydrogen di-phosphates into normal phosphate. If we designate this by a, and the quantity of total P_2O_5 in milligrammes in the same quantity of urine, as determined by a method which will be described later, by g, then we obtain the quantity of P_2O_5 in milligrammes in the di-hydrogen phosphate s by the following formula: $s = 17.75a - g$.

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[1] Zeitschr. f. klin. Med., Bd. 7, Suppl., 1884.

[2] Maly, Zeitschr. f. anal. Chem., Bd. 15, and F. Hoffmann, Arch. d. Heilkunde, Bd. 17.

If, for example, in a case in which the conversion of both phosphates into normal phosphate in 100 c. c. of the urine required 20 c. c. caustic soda, while the total quantity of P_2O_5 in 100 c. c. urine was 275 milligrammes, then $s = 17.75 \times 20 - 275 = 80$ milligrammes. The quantity of P_2O_5 as simple acid phosphate was therefore 195 milligrammes.

This method, according to Lieblein,[1] gives too high figures for the di-hydrogen phosphate. The quantity of alkali used is too great because of the formation of basic barium phosphate. Lieblein recommends the following method as suggested by Freund.[2] First determine the total quantity of phosphoric acid in the urine by means of titration with uranium solution and then precipitate the phosphoric acid existing as simple acid salts in another portion by barium chloride, and determine the phosphoric acid remaining in a portion of the filtrate as monophosphate by titration with uranium solution.

According to Lieblein 10 c. c. of a normal barium chloride solution (122 grm. $BaCl_2, 2H_2O$ in 1 litre) are used to precipitate each 100 milligrammes total phosphoric acid existing as simple acid salts, the filtrate made up to 100 c. c. and the phosphoric acid determined in 50 c. c. thereof. In the precipitation of the urine with $BaCl_2$ about 3% of the phosphoric acid existing as simple acid salts remains in solution as double acid salt, and hence a corresponding correction must be made. As one third of the phosphoric acid is combined with fixed bases as double acid salts, Lieblein is of the opinion that in calculating the acidity of a urine only two thirds of this phosphoric acid is to be ascribed thereto.

Freund and Toepfer[3] have lately suggested a method for the determination of the acidity as well as the alkalinity of the urine by means of titration with $\frac{n}{10}$ caustic soda, or $\frac{n}{10}$ hydrochloric acid, using phenolphthalein, sodium alizarin sulphonate, or a solution of Poirier's blue as indicators. Lieblein's[4] investigations do not speak in favor of this method.

A urine with an alkaline reaction caused by fixed alkalies has a very different diagnostic value from one whose alkaline reaction is caused by the presence of ammonium carbonate. In the latter case we have to deal with a decomposition of the urea of the urine by the action of micro-organisms.

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[1] Zeitschr. f. physiol. Chem., Bd. 20.

[2] Centralbl. f. d. med. Wissensch., 1892, S. 689.

[3] Zeitschr. f. physiol. Chem., Bd. 19, S. 84.

[4] L. c.

If we wish to determine whether the alkaline reaction of the urine is due to ammonia or fixed alkalies, we dip a piece of red litmus-paper into the urine and allow it to dry exposed to the air or to a gentle heat. If the alkaline reaction is due to ammonia, the paper becomes red again; but if it is caused by fixed alkalies, it remains blue.

The **specific gravity** of urine, which is dependent upon the relationship existing between the quantity of water secreted and the solid urinary constituents, especially the urea and sodium chloride, may vary considerably, but is generally 1.017–1.020. After drinking large quantities of water it may fall to 1.002, while after profuse perspiration or after drinking very little water it may rise to 1.035–1.040. In new-born infants the specific gravity is low, 1.007–1.005. The determination of the specific gravity is an important means of learning the average amount of solids eliminated from the organism with the urine, and on this account the determination becomes of true value only when at the same time the quantity of urine voided in a given time is determined. The different portions of urine voided in the course of the 24 hours are collected, mixed together, the total quantity measured, and then the specific gravity taken.

The *determination of the specific gravity* is most accurately obtained with the pyknometer. For ordinary cases the specific gravity may be determined with sufficient accuracy by means of areometers. The areometers found in the trade, or *urinometers*, are graduated from 1.000 to 1.040; for exact observations it is better to use two urinometers, one graduated from 1.000 to 1.020, and the other from 1.020 to 1.040. A special urinometer is that of HELLER, which is graduated according to Baumé's scale, from 0 to 8. Each degree corresponds to 7 degrees of the ordinary urinometer, and as the zero-point of HELLER's urinometer corresponds to the figure 1000, then the 1, 1.5, 2, 2.5, 3, etc., degrees of HELLER's urinometer correspond to 1.007, 1.0105, 1.014, 1.0175, 1.024, etc., of the ordinary specific gravity.

To determine the specific gravity of urine, if necessary filter the urine, or if it contains a urate sediment, first dissolve it by gentle heat, then pour the clear urine into a dry cylinder, avoiding the formation of froth. Air-bubbles or froth, when present, must be removed with a glass rod or filter-paper. The cylinder, which must be about ⅘ full, must be wide enough to allow the urinometer to swim freely in the liquid without touching the sides. The cylinder and urinometer should both be dry or previously washed with the urine. On reading, the eye is brought on a level with the lower meniscus—which occurs when the surface of the liquid and the lower limb of the meniscus coincide; the reading is then made from the point where this curved line cuts the scale of the urinometer. If the eye is not in the same horizontal plane with the convex line of the meniscus, but is too high or too low, the surface of the liquid assumes the shape of an ellipse, and the reading in this position is incorrect. Before reading press the urinometer gently down into the liquid and then allow it to rise, and wait until it is at rest.

If the quantity of urine at disposal is not sufficient to fill the cylinder to the proper height it may be diluted, according to circumstances, with an equal volume or several volumes of water. This does not give quite accurate results, and with small quantities of urine it is best to determine the specific gravity by means of the pyknometer.

Each urinometer is graduated for a certain temperature, which is marked on the instrument, or at least on the best. If the urine is not at the proper temperature, the following corrections must be made: For every three degrees above the normal temperature one unit of the last order is added to the reading, and for every three degrees below the normal temperature one unit (as above) is subtracted from the specific gravity observed. For example, when a urinometer graduated for + 15° C. shows a specific gravity of 1.017 at + 24° C., then the specific gravity at + 15° C. = 1.017 + 0.003 = 1.020.

II. Organic Physiological Constituents of the Urine.

Urea, $\overset{+}{\mathrm{U}}$r, which is ordinarily considered as carbamid, $CO(NH_2)_2$, may be synthetically prepared in several different ways, namely, from carbonyl-chloride, or carbonic-acid ethyl-ether and ammonia, $COCl_2 + 2NH_3 = CO(NH_2)_2 + 2HCl$, or $(C_2H_5)_2.O_2.CO + 2NH_3 = 2(C_2H_5.OH) + CO(NH_2)_2$; by the metameric decomposition of ammonium cyanate, $CO:N.NH_4 = CO(NH_2)_2$ (Wöhler, 1828); and in many other ways. It is also formed by the decomposition or oxidation of certain bodies found in the animal organism, such as creatin and uric acid.

Urea is found most abundant in the urine of carnivora and ma but in smaller quantities in that of herbivora. The quantity i human urine is ordinarily 20–30 p. m. It has also been found i small quantities in the urine of certain birds and amphibian Urea occurs in the perspiration in small quantities, and as traces i the blood and in most of the animal fluids. It also occurs in rath large quantities in the blood, liver, muscle (v. Schroeder[1]) an bile[2] of sharks. Urea is also found in certain tissues and organs mammals, especially in the liver and spleen, and in smaller quantities in the muscles. Under pathological conditions, as in obstructed excretion, urea may appear to a considerable extent in the animal fluids and tissues.

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[1] Zeitschr. f. physiol. Chem., Bd. 14.

[2] Investigations not published by the author.

The quantity of urea which is voided in 24 hours on a mixed diet is in a grown man about 30 grms., for women somewhat less. Children void absolutely less, but relative to their body-weight the excretion is larger than in grown persons. The physiological significance of urea lies in the fact that this body forms in man and carnivora, from a quantitative standpoint, the most important nitrogenous final product of the metabolism of proteid bodies. On this account the elimination of urea varies to a great extent with the amount of proteid transformed, and above all with the quantity of absorbable proteids in the food taken. The elimination of urea is greatest after an exclusive meat diet, and lowest, indeed less than during starvation, after the consumption of non-nitrogenous bodies, for these diminish the metabolism of the proteids of the body.

If the consumption of the proteids of the body is increased, then the elimination of urea is correspondingly increased. This is found to a rather high degree in certain diseases with fever: also in other cases of increased elimination of nitrogen, such as after poisoning with arsenic, antimony, and phosphorus, by a diminished supply of oxygen—as in severe and continuous dyspnœa, poisoning with carbon monoxide, hemorrhage, etc.—it used to be considered that it was due to an increased elimination of urea because no exact difference was made between the quantity of urea and the total quantity of nitrogen in the urine. Recent researches have completely demonstrated the untrustworthiness of these observations. Since PFLÜGER and BOHLAND[1] have shown that 16% of the total nitrogen of the urine exists under physiological conditions as other combinations, not urea, attention has been called to the relative relationship of the different nitrogenous constituents of the urine to each other, and it has been found, under pathological conditions, that this relationship may vary very considerably, especially in regard to the urea. We have numerous estimations by different investigators, such as BOHLAND,[2] E. SCHULTZE,[3] CAMERER,[4]

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[1] Pflüger's Arch., Bdd. 38 and 43.

[2] *Ibid.*, Bd. 43.

[3] *Ibid.*, Bd. 45.

[4] Zeitschr. f. Biologie, Bdd. 24, 27, and 28.

VOGES,[1] MÖRNER and SJÖQVIST,[2] GUMLICH,[3] and others, on the relationship of the different nitrogenous constituents to each other in normal urine of adults. SJÖQVIST[4] has made similar estimations on new-born babes from 1–7 days old. From all these analyses we obtain the following figures, A for adults and B for new-born babes. Of the total nitrogen, we have:

	A	B
Urea	84–91%	73–76
Ammonia	2–5	7.8–9.6
Uric acid	1–3	3.0–8.5
Remaining nitrogenous substances (extractives)	7–12	7.3–14.7

The different relationship between uric acid, ammonia, and urea nitrogen in children and adults is remarkable, since the urine of children is considerably richer in uric acid and ammonia and considerably poorer in urea than the urine of adults. In disease the proportion of the nitrogenous substances may be markedly changed and a decrease in the quantity of urea and an increase in the quantity of ammonia have been observed in certain diseases of the liver. This will be treated of in detail in connection with the formation of urea in the liver. It is natural that there is a diminished formation of urea in diminished administration of proteids or diminished consumption of proteids. In diseases of the kidneys which disturb or destroy the integrity of the epithelium of the tortuous urinary passage the elimination of urea is considerably diminished.

Formation of urea in the organism. The experiments to produce urea directly from proteids by oxidation have not led to any positive results. On the contrary DRECHSEL, as mentioned in Chapter II, has obtained lysin and lysatin as products of the hydrolytic cleavage of proteids and obtained urea from the lysatin by the action of alkalies. According to DRECHSEL and HEDIN (see Chap. II, p. 21, and Chap. IX, p. 302) these two bodies are produced by the hydrolytic cleavage of proteids by trypsin, and it is also possible that a part of the urea may be formed by a hydrolytic cleavage of proteids with these two bodies as intermediate steps.

Creatin and creatinin, which are homologues of lysatin, are products of the destruction of proteid in the animal body and also yield urea by the action of alkalies, hence they may be steps in the formation of urea in the body.

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[1] Ueber die Mischung der stickstoffhaltigen Bestandtheile im Harn, etc. Inaug.-Diss. Berlin, 1892. Cited from Maly's Jahresber., Bd. 22.

[2] Skand. Arch. f. Physiol., Bd. 2. See also Sjöqvist, Nord. med. Arkiv, 1892, No. 36.

[3] Zeitschr. f. physiol Chem., Bd. 17.

[4] Nord. med. Arkiv., 1894, No. 10.

In the decomposition of proteid bodies we ordinarily obtain, as mentioned in Chapter II, amido-acids of various kinds, hence we consider the amido-acids as intermediate steps in the formation of urea from proteids. It has been shown that leucin and glycocoll (SCHULTZEN and NENCKI,[1] SALKOWSKI[2]) and aspartic acid (v. KNIERIEM[3]) may be in part transformed into urea within the organism. The nature of the chemical processes by which these transformations are effected is not positively known. SCHMIEDEBERG claims that the nitrogenous combinations in which the nitrogen exists in the group NH_2-CH_2 are decomposed in the organism with the formation of ammonia, and also that the ammonium carbonate is then converted by a synthesis into urea. The correctness of the last statement has been recently confirmed by many investigators. Thus the researches of v. KNIERIEM,[4] SALKOWSKI,[5] FEDER,[6] I. MUNK,[7] CORANDA,[8] SCHMIEDEBERG and FR. WALTER,[9] and HALLERWORDEN,[10] on the behavior of ammonium salts in the animal body and the elimination of the ammonia under various conditions, have shown that the ammonium salts with strong acids act differently in the organism of carnivora and herbivora, while ammonium carbonate or such salts which are burnt into carbonate in the organism are transformed into urea by carnivora as well as herbivora. The researches of v. SCHRÖDER[11] have given an explanation as to the organ in which urea is formed. By passing blood which had been treated with ammonium carbonate or ammonium formate through a dog's liver he found a very considerable formation of urea, and these observations have been confirmed by the very careful observations of SALOMON.[12] The formation of urea from ammonium carbonate is to be considered as a synthesis with the expulsion of water.

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[1] Zeitschr. f. Biologie, Bd. 8.

[2] Zeitschr. f. physiol. Chem., Bd. 4.

[3] Zeitschr. f. Biologie, Bd. 10.

[4] *Ibid.*, Bd. 10.

[5] Zeitschr. f. physiol. Chem., Bd. 1.

[6] Zeitschr. f. Biologie, Bd. 13.

[7] Zeitschr. f. physiol. Chem,, Bd. 2.

[8] Arch. f. Path. u. Pharm., Bd. 12.

[9] *Ibid.*, Bd. 7.

[10] *Ibid.*, Bd. 10.

[11] *Ibid.*, Bd. 15.

[12] Virchow's Arch., Bd. 97.

The formation of urea from amido-acids has been explained in other ways. SCHULTZEN and NENCKI[1] have expressed the view that the amido-acids yield carbamic acid in the animal body, which then is transformed into urea. This view has later received further support by more important observations. DRECHSEL[2] has shown that the amido-acids yield carbamic acids by oxidation in alkaline fluid outside of the organism, and he obtained urea from ammonium carbamate by passing an alternate electric current through its solution, namely, by alternate oxidation and reduction. DRECHSEL has also been able to detect small quantities of carbamates in blood, and later in conjunction with ABEL[3] he detected carbamic acid in alkaline horse's urine. DRECHSEL therefore accepts the formation of urea from ammonium carbamate, and according to him the alternating oxidation and reduction take place in the following way:

$$H_4N.O.CO.NH_2 + O = H_2N.O.CO.NH_2 + H_2O$$

and

$$H_2N.O.CO.NH_2 + H_2 = \underset{\text{Urea.}}{H_2N.CO.NH_2} + H_2O.$$

ABEL and MUIRHEAD[4] have later observed an abundant elimination of carbamic acid in human and dog's urine after the administration of large quantities of milk of lime, and finally the regular appearance of this acid in normal acid human and dog's urine has been made very probable by M. NENCKI and HAHN.[5] These last-mentioned investigators have also given very important support to the theory of the formation of urea from ammonium carbamate by observations on dogs with ECK'S fistula. In this case the portal vein is directly connected with the inferior vena cava, and a communication is thus established so that the blood of the portal vein flows directly into the vena cava, without passing through the liver. NENCKI and HAHN observed violent symptoms of poisoning in dogs after this operation, and these symptoms were quite identical with those obtained on introducing carbamate into the blood. These symptoms also appear after the introduction of carbamate into the stomach, while the introduction of carbamate into the stomach of a normal dog had no action. As these observers also found that the urine of the dog on which the operation was made was richer in carbamate than that of the normal dog, they conclude that the symptoms were due to the non-transformation of the ammonium carbamate into urea in the liver, and they consider the ammonium carbamate as the substance from which the urea is derived in the liver of mammals.

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[1] Zeitschr. f. Biologie, Bd. 8.

[2] Ber. d. sächs. Gesellsch. d. Wissensch., 1875. See also Journ. f. prakt. Chem. (N. F.), Bdd. 12, 16, and 22.

[3] Du Bois-Reymond's Arch., 1891, S. 236.

[4] Arch. f. exp. Path. u. Pharm., Bd. 31.

[5] Hahn, Massen, Nencki et Pawlow, La fistule d'Eck de la veine cav[illegible] férieur et de la veine porte, etc. Arch. des sciences biol. de St. Pétersbo[illegible] Tome 1, No. 4, 1892; also Arch. f. exp. Path. u. Pharm., Bd 32, S. 161.

The view as to the formation of urea from ammonium carbamate does not contradict the above statement as to the transformation of carbonates into urea, since we can imagine that the carbonate is first converted into carbamate with the expulsion of a molecule of water, and that this then is transformed into urea with the expulsion of a second molecule of water.

Besides the above-mentioned theories as to the formation of urea we have others, which will not be given because the only theory which has thus far been positively demonstrated is the formation of urea from ammonium compounds in the liver.

The question in which organ urea is formed has been the subject of numerous investigations. From the researches of numerous investigators, PREVOST and DUMAS, MEISSNER, VOIT, GRÉHANT, GSCHEIDLEN, SALKOWSKI, and v. SCHRÖDER,[1] it has been found that the extirpation of the kidneys causes a considerable increase in the quantity of urea in the blood, and that the kidneys therefore, if they produce urea at all, are not the only organs which can produce it. By experiments performed on the removed kidneys, which were analogous to the above-mentioned experiments on the removed liver, v. SCHRÖDER has shown that neither the kidneys nor the muscles nor the remaining tissues of the lower extremities of the dog have the property of forming urea from ammonium carbonate. The liver is the only organ where the formation of urea from ammonium compounds has been proved with certainty, and the question arises as to the importance of these compounds to the urea synthesis in the liver. Is all or the chief mass of the urea formed from ammonium compounds in the liver?

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[1] Arch. f. exp. Path. u. Pharm., Bdd. 15 and 19. In regard to the above-cited researches and the older literature on this subject we refer the reader to v. Schröder, and also Voit, Zeitschr. f. Biologie, Bd. 4.

No satisfactory answer can be given at present to this question. If urea is formed from ammonium combinations in the liver, then we can expect a diminished or reduced formation of urea and a corresponding increase in the elimination of ammonia in extirpation of the liver. The normal relationship between ammonia and urea in the urine must in these cases be essentially changed. In order to demonstrate this, experiments have been made on animals, and the urine in men with liver disease has been examined.

The extirpation and atrophy experiments on animals made by different methods by NENCKI and HAHN,[1] SLOSSE,[2] and LIEBLEIN[3] have shown that a rather marked increase of ammonia and a diminished elimination of urea take place after the operation, but also that there are cases in which, irrespective of the pronounced atrophy, an abundant formation of urea takes place and no appreciable if any change in the proportion of ammonia to the total nitrogen and urea is observed.

The observations on human beings with diseases of the liver lead to similar results. In this regard the numerous investigations of HALLERWORDEN,[4] STADELMANN,[5] FRÄNKEL,[6] FAWITZKI,[7] MÖRNER and SJÖQVIST,[8] GUMLICH,[9] v. NOORDEN,[10] WEINTRAUD,[11] MÜNZER,[12] and WINTERBERG[13] and others on the urine in cirrhosis of the liver, acute yellow atrophy of the liver, and phosphorus poisoning, are available. We learn from these investigations that in certain cases the proportion of the nitrogenous substances may be so changed that urea is only 50–60% of the total nitrogen, while in other cases, on the contrary, even in very extensive atrophy of the liver-cells, the formation of urea is not diminished, neither is the proportio between the total nitrogen, urea, and ammonia essentially changed. Even in the cases in which the formation of urea was relatively diminished and the elimination of ammonia considerably increased we must not without further investigation assume a reduced ability of the organism to produce urea. An increased elimination of ammonia may, as shown by MÜNZER in the case of acute phosphorus poisoning, be dependent upon the formation of abnormally large quantities of acids, caused by abnormal metabolism, and these acids require a greater quantity of ammonia for their neutralization.

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[1] L. c.

[2] Du Bois-Reymond's Arch., 1890.

[3] Arch. f. exp. Path. u. Pharm., Bd. 32.

[4] Arch. f. exp. Path. u. Pharm., Bd. 12.

[5] Deutsch. Arch. f. klin. Med., Bd. 33.

[6] Berlin klin. Wochenschr., Jahrg. 1878 and 1892.

[7] Deutsch. Arch. f. klin. Med., Bd. 45.

[8] Skand. Arch. f. Physiol., Bd. 2; see also Sjöqvist, Nord. med. Arkiv., Jahrg. 1892, No. 36.

[9] Zeitschr. f. physiol. Chem., Bd. 17.

[10] Lehrb. d. Pathol. des Stoffwechsels, S. 287.

[11] Arch. f. exp. Path. u. Pharm., Bd. 31.

[12] Deutsch. Arch. f. klin. Med., Bd. 52.

[13] Münzer and Winterberg, Arch. f. exp. Path. u. Pharm., Bd. 33.

For the present we are not justified in the statement that the liver is the only organ in which urea is formed, and continued investigation only can yield further information as to the extent and importance of the formation of urea from ammonia compounds in the liver.

Properties and Reactions of Urea. Urea crystallizes in needles or in long, colorless, four-sided, often hollow, anhydrous rhombic prisms. It has a neutral reaction and produces a cooling sensation on the tongue like saltpetre. It melts at 130–132° C., but decomposes already at about 100° C. At ordinary temperatures it dissolves in equal weight of water and in five parts alcohol; it requires one part boiling alcohol for solution; it is insoluble in alcohol-free ether and also in chloroform. If urea in substance is heated in a test-tube, it melts, decomposes, gives off ammonia, and leaves finally a non-transparent white residue which, among other substances, contains also cyanuric acid and *biuret*, which dissolves in water, giving a beautiful reddish-violet liquid with copper sulphate and alkali (*biuret reaction*). On heating with baryta-water or caustic alkali, also in the so-called alkaline fermentation of urine caused by micro-organisms, urea splits into carbon dioxide and ammonia with the addition of water. The same decomposition products are produced when urea is heated with concentrated sulphuric acid. An alkaline solution of sodium hypobromite decomposes urea into nitrogen, carbon dioxide, and water according to the equation

$$CON_2H_4 + 3NaOBr = 3NaBr + CO_2 + 2H_2O + N_2.$$

With a concentrated solution of furfurol and hydrochloric acid urea in substance gives a coloration passing from yellow, green, blue to violet and then beautiful purple-violet after a few minutes

(SCHIFF'S[1] reaction). According to HUPPERT[2] the test is best performed by taking 2 c. c. of a concentrated furfurol solution, 4–6 drops concentrated hydrochloric acid, and adding to this mixture, which must not be red, a small crystal of urea. A deep violet coloration appears in a few minutes.

Urea forms crystalline combinations with many acids. Among these the one with nitric acid and the one with oxalic acid are the most important.

UREA NITRATE, $CO(NH_2)_2.HNO_3$. On crystallizing quickly this combination forms thin rhombic or six-sided overlapping tiles, colorless plates, whose point has an angle of 82°. When crystallizing slowly, larger and thicker rhombic pillars or plates are obtained. This combination is rather easily soluble in pure water, but is considerably less soluble in water containing nitric acid; it may be obtained by treating a concentrated solution of urea with an excess of strong nitric acid free from nitrous acid. On heating this combination it volatilizes without leaving a residue.

This compound may be employed with advantage in detecting small amounts of urea. A drop of the concentrated solution is placed on a microscope-slide and the cover-glass placed upon it; a drop of nitric acid is then placed on the side of the cover-glass and allowed to flow under. The formation of crystals begins where the solution and the nitric acid meet. Alkali nitrates may crystallize very similarly to urea nitrate when they are contaminated with other bodies; therefore, in testing for urea, the crystals must be identified as urea nitrate by heating and by other means.

UREA OXALATE, $2.CO(NH_2)_2.H_2C_2O_4$. This compound is more sparingly soluble in water than the nitric-acid compound. It is obtained in rhombic or six-sided prisms or plates on adding a saturated oxalic-acid solution to a concentrated solution of urea.

Urea also forms combinations with mercuric nitrate in variable proportions. If a very faintly acid mercuric-nitrate solution is added to a two-per-cent solution of urea and the mixture carefully neutralized, a combination is obtained of a constant composition which contains for every 10 parts of urea 72 parts mercuric oxide. This compound serves as the basis of LIEBIG'S titration method. Urea combines also with salts, forming mostly crystallizable combinations, as, for instance, with sodium chloride, with the chlorides of the heavy metals, etc. An alkaline but not a neutral solution of urea is precipitated with mercuric chloride.

The method of preparing urea from urine is chiefly as follows
Concentrate the urine, which has been faintly acidified with su

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[1] Ber. d. deutsch. chem. Gesellsch., Bd. 10.

[2] Huppert-Neubauer, Analyse des Harnes, 10. Aufl., S. 296.

phuric acid, at a low temperature, add an excess of nitric acid, at the same time keeping the mixture cool, press the precipitate well, decompose it in water with freshly precipitated barium carbonate, dry on the water-bath, extract the residue with strong alcohol, decolorize when necessary with animal charcoal, and filter while warm. The urea which crystallizes on cooling is purified by recrystallization from warm alcohol. A further quantity of urea may be obtained from the mother-liquor by concentration. The urea is purified from contaminating mineral bodies by redissolving in alcohol-ether. If it is only necessary to detect the presence of urea in urine, it is sufficient to concentrate a little of the urine on a watch-glass and after cooling treat with an excess of nitric acid. In this way we obtain crystals of urea nitrate.

Quantitative Estimation of Urea in urine. The methods suggested for this purpose are those of LIEBIG by titration, of HEINTZ and RAGSKY, also that of KJELDAHL, by which the total nitrogen is determined, and those of BUNSEN and KNOP-HÜFNER and MÖRNER-SJÖQVIST, where urea is intended to be determined as such. Among these methods, that of LIEBIG, which is perhaps the one most frequently employed by physicians, and that of MÖRNER-SJÖQVIST will here be carefully explained. In regard to the others, whose chief points only will be spoken of here, the student is referred to other text-books.

LIEBIG'S METHOD is based upon the fact that a dilute solution of mercuric nitrate under proper conditions precipitates all the urea, forming a compound of constant composition. As indicator, a soda solution or a thin paste of sodium bicarbonate is used. An excess of mercuric nitrate produces herewith a yellow or yellowish-brown combination, while the combination of urea and mercury is white. PFLÜGER[1] has given full particulars of this method; therefore we will describe PFLÜGER's modification of LIEBIG's method.

As phosphoric acid is also precipitated by the mercuric-nitrate solution, this must be removed from the urine by the addition of a baryta solution before titration. PFLÜGER also suggested that the acidity produced by the mercury solution be neutralized during titration by the addition of a soda solution. The liquids necessary for the titration are the following:

1. *Mercuric Nitrate Solution.* This solution is calculated for a 2% urea solution, and 20 c. c. of the first should correspond to 10 c. c. of the latter. Each c. c. of the mercury solution corresponds to 0.01 grm. urea. As a small excess of HgO is necessary in the urine to make the final reaction (with alkali carbonate or bicarbonate) appear, each c. c. of the mercury solution must contain

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[1] Pflüger, and Pflüger and Bohland, in Pflüger's Arch., Bdd. 21, 36, 37, and 40.

0.0772 instead of 0.0720 grm. HgO. The mercury solution contains therefore 77.2 grms. HgO in one litre.

The solution may be prepared from pure mercury or mercuric oxide by dissolving in nitric acid. The solution, freed as completely as possible from an excess of acid, is diluted by the careful addition of water, stirring meanwhile, until it has a specific gravity of 1.10 or a little higher at + 20° C. The solution is standardized with a 2% solution of pure urea which has been dried over sulphuric acid, and the operation conducted as will be described later. If the solution is too concentrated, it is corrected by the careful addition of the necessary amount of water, avoiding precipitation of basic salt, and titrating again. The solution is correct if 19.8 c. c. of it added at once to 10 c. c. of the urea solution and the necessary quantity of normal soda solution (11–12 c. c. or more) to nearly completely neutralize the liquid, gives the final reaction when exactly 20 c. c. of the mercury solution have been employed.

2. *Baryta Solution.* This consists of 1 vol. barium-nitrate and 2 vols. barium-hydrate solution, both saturated at the ordinary temperature.

3. *Normal Soda Solution.* This solution contains 53 grms. pure anhydrous sodium carbonate in 1 litre of water. According to PFLÜGER a solution having a specific gravity of 1.053 is sufficient. The amount of this soda solution necessary to completely neutralize the acid set free during the titration is determined by titrating with a pure 2% urea solution. To facilitate operations a table can be made showing the quantity of soda solution necessary when from 10 to 35 c. c. of the mercury solution is used.

Before the titration the following must be considered. The chlorides of the urine interfere with the titration in that a part of the mercuric nitrate is transformed into mercuric chloride, which does not precipitate the urea. The chlorides of the urine are therefore removed by a silver-nitrate solution, which also removes any bromine or iodine combinations which may exist in the urine. If the urine contains proteid in noticeable amounts, it must be removed by coagulation and the addition of acetic acid, but care must be taken that the concentration and the volume of the urine is not changed during these operations. If the urine contains ammonium carbonate in notable quantities, caused by alkaline fermentation, this titration method cannot be applied. The same is true of urine containing leucin, tyrosin, or medicinal preparations precipitated by mercuric nitrate.

In cases where the urine is free from proteid or sugar and not specially poor in chlorides, the quantity of urea, and also the approximate quantity of mercuric nitrate necessary for the titration, may be learned from the specific gravity. A specific gravity of 1.010 corresponds to about 10 p. m., a specific gravity of 1.01 generally somewhat less than 15 p. m., and a specific gravity of 1.015-1.020 about 15–20 p. m. urea. With a specific gravity higher than 1.020 the urine generally contains more than 20 p. m. of urea, and above this point the amount of urea increases much more rapidly than the specific gravity, so that with a specif gravity of 1.030 it contains over 40 p. m. urea. Fever urines with a specific gravity above 1.020 sometimes contain 30–40 p. m. urea, or even more.

PREPARATION FOR THE TITRATION. If a large amount of urea is suspected from a high specific gravity, the urine must first be diluted with a carefully measured quantity of water, so that the amount of urea is reduced below 30 p. m. In a special portion of the same urine the amount of chlorides is determined by one of the methods which will be given later, and the number of c. c. of silver-nitrate solution necessary is noted. Then a larger quantity of urine, say 100 c. c., is mixed with one half or, if this is not sufficient to precipitate all the sulphuric and phosphoric acids, with an equal volume of the baryta solution; it is then allowed to stand a little while, and the precipitate is filtered through a dried filter. From the filtrate containing the urine diluted with water a proper quantity, corresponding to about 60 c. c. of the original urine, is measured, and exactly neutralized with nitric acid added from a burette, so that the exact quantity employed is known. The neutralized mixture of urine and baryta is treated with the proper quantity of silver-nitrate solution necessary to completely precipitate the chlorides, which was ascertained by a previous determination. The mixture containing a known volume of urine is now filtered through a dried filter into a flask, and from the filtrate an amount is measured corresponding to 10 c. c. of the original urine.

EXECUTION OF THE TITRATION. Nearly the total quantity of mercuric-nitrate solution to be used, and which is known from the specific gravity of the urine, is added at once, and immediately afterwards the quantity of soda solution necessary, as indicated by the table. If the mixture becomes yellowish in color, then too much mercury solution has been added and another determination must be made. If the test remains white, and if a drop taken out and placed on a glass plate with a dark background and stirred with a drop of a thin paste of sodium bicarbonate does not give a yellow color, the addition of mercury solution is continued by adding $\frac{1}{2}$ and then $\frac{1}{10}$ c. c., and testing after each addition in the following way: A drop of the mixture is placed on a glass plate with a dark background beside a small drop of the bicarbonate paste. If the color after stirring the two drops together is still white after a few seconds, then more mercury solution must be added; if, on the contrary, it is yellowish, then—if not too much mercury solution has been added by inattention—the result to $\frac{1}{10}$ c. c. has been found. By this approximate determination, which is sufficient in many cases, we have fixed the minimum amount of mercury solution necessary to add to the quantity of urine in question, and we now proceed to the final determination.

A second quantity of the filtrate, corresponding to 10 c. c. of the original urine, is filtered, and the same quantity of mercury solution added at one time as was found necessary to produce the

the phosphoric acid. After cooling, an excess of caustic soda is added and the ammonia distilled into a titrated acid, which must then be retitrated. After subtracting the quantity of pre-existing ammonia very accurate results are obtained for the ammonia originating from the urea (and perhaps from an unknown ureid present in the urine).

KNOP-HÜFNER'S METHOD[1] is based on the fact that urea by the action of sodium hypobromite splits into water, carbon dioxide (which dissolves in the alkali), and nitrogen, whose volume is measured (see page 459). This method is less accurate than the preceding ones, and therefore in scientific work it is discarded. It is of value to the physician and for practical purposes because of the ease and rapidity with which it may be performed, even though it may not give very accurate results. For practical purposes a series of different apparatus have been constructed to facilitate the use of this method.[2] Among these ESBACH'S *ureometer* deserves to be especially mentioned. In regard to the reagents necessary for the determination of urea, and also for instructions in the use of this instrument, we must refer the reader to the directions accompanying the apparatus. For pure urea solutions ESBACH'S apparatus gives quite exact results. The determination of urea in urine by this method always gives results somewhat too low, and as a rule a result is obtained which on an average is about 0.1% lower than that obtained with LIEBIG'S titration method.

MÖRNER-SJÖQVIST METHOD.[3] According to this method the nitrogenous constituents of the urine, with the exception of the urea and ammonia, are first precipitated by alcohol-ether after the addition of a solution of barium chloride and barium hydrate and then the urea determined in the concentrated filtrate, after driving off the ammonia, by KJELDAHL'S nitrogen estimation.

The procedure is as follows: Mix 5 c. c. of the urine in a flask with 5 c. c. saturated $BaCl_2$ solution, in which 5% barium hydrate is dissolved. Then add 100 c. c. of a mixture of two parts 97% alcohol and 1 part ether and allow this to stand in the closed flask overnight. The precipitate is filtered off and washed with alcohol-ether. The alcohol and ether is removed from the filtrate by distillation at about 55° C. (not above 60° C.). When the liquid is reduced to about 25 c. c. a little water and calcined magnesia are added and the evaporation continued until the vapors are no longer alkaline in reaction, which generally is found before it is concentrated to 15–10 c. c. This concentrated liquid is transferred into a proper flask by the aid of a little water, treated with a few drops of concentrated sulphuric acid and further concentrated on the water-bath. Now 20 c. c. pure concentrated sulphuric acid are added and the process carried out according to KJELDAHL. According to BÖDTKER[1] the addition of magnesia is unnecessary, and it is best to avoid it entirely as it easily leads to a small loss of urea. This exact method is to be recommended.

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[1] Knop, Zeitschr. f. analyt. Chem., Bd. 9; Hüfner, Jour. f. prakt. Chem. (N. F.), Bd. 3. See also Huppert-Neubauer, 10. Aufl.

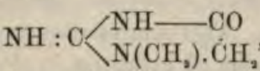
[2] See Huppert-Neubauer.

[3] Skand. Arch. f. Physiol., Bd. 2.

Carbamic Acid, $H_2N.COOH$. This acid is not known in the free state, but only as salts. Ammonium carbamate is produced by the action of dry ammonia on dry carbon dioxide. Carbamic acid is also produced by the action of potassium permanganate on proteid and several other nitrogenous organic bodies.

We have already spoken of the occurrence of carbamic acid in human and animal urines in connection with the formation of urea. The calcium salt, which is soluble in water and ammonia but insoluble in alcohol, is most important in the detection of this acid. The solution of the calcium salt in water becomes cloudy on standing, but much quicker on boiling, and calcium carbonate separates.

Carbamic acid ethylester (urethan), as shown by JAFFÉ,[2] may pass, by the mutual action of alcohol and urea, into the alcoholic extract of the urine when working with large quantities of urine.

Creatinin, $C_4H_7N_3O$, or  $NH : C\left\langle\begin{array}{l}NH\text{——}CO\\ N(CH_3).CH_2\end{array}\right.$, is generally considered as the anhydride of creatin (see page 366) found in the muscles. It occurs in human urine and in that of certain mammalia. It has also been found in ox-blood, milk, though in very small amounts, and in the flesh of certain fishes. According to JOHNSON[3] a creatinin occurs in fresh ox-flesh which differs from that occurring in urine and from which the creatin of the muscles is formed by bacterial action.

The quantity of creatinin in human urine is for a grown man, voiding a normal quantity of urine in the 24 hours, 0.6–1.3 grms. (NEUBAUER[4]), or on an average 1 grm. The quantity is dependent on the food, and decreases in starvation. Sucklings do not generally eliminate any creatinin, and it only appears in the urine when the milk is replaced by other food. The quantity of creatinin in urine varies as a rule with the quantity of urea, although it is increased more by flesh (because the flesh contains creatin) than by proteid. GROCCO[1] and MOITESSIER[2] claim that the elimination of creatinin is increased by muscular activity. The behavior of creatinin in disease is little known. By increased metabolism the amount is increased, while by decreased exchange of material, as in anæmia and cachexia, it is diminished.

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[1] Zeitschr. f. physiol. Chem., Bd. 17.

[2] *Ibid.*, Bd. 14.

[3] Proc. Roy. Soc., Vol. 50. Cited from Maly's Jahresber., Bd. 22.

[4] Huppert-Neubauer, Harnanalyse, 10. Aufl., S. 387.

Creatinin crystallizes in colorless, shining monoclinic prisms which differ from creatin crystals in not becoming white with loss of water when heated to 100° C. It dissolves in 11.5 parts cold water, but more easily in warm water. It requires nearly 100 parts cold absolute alcohol for solution,[3] but it is more soluble in warm alcohol. It is nearly insoluble in ether. In alkaline solution creatinin is converted into creatin very easily on warming.

Creatinin gives an easily soluble crystalline combination with hydrochloric acid. A solution of creatinin acidified with mineral acids gives crystalline precipitates with phospho-tungstic or phospho-molybdic acids even in very dilute solutions (1 : 10,000) (KERNER,[4] HOFMEISTER[5]). It is precipitated, like urea, by mercuric-nitrate solution. Among the compounds of creatinin, that with zinc chloride, *creatinin zinc-chloride*, $(C_4H_7N_3O)_2ZnCl_2$, is of special interest. This combination is obtained when a sufficiently concentrated solution of creatinin in alcohol is treated with a concentrated, faintly acid solution of zinc chloride. Free mineral acids dissolve the combination, hence they must not be present; this, however, may be prevented, when they are present, by an addition of sodium acetate. In the impure state, as ordinarily obtained from urine, creatinin zinc chloride forms a sandy, yellowish powder which under the microscope appears as fine needles forming concentric groups, mostly complete rosettes or yellow balls or tufts, or grouped as brushes. On slowly crystallizing, or when very pure, more sharply defined prismatic crystals are obtained. This combination is sparingly soluble in water.

Creatinin acts as a reducing agent. Mercuric oxide is reduced to metallic mercury, and oxalic acid and methylguanidin (methyluramin) are formed. Creatinin also reduces copper hydroxide in alkaline solution, forming a colorless soluble combination, and only after continuous boiling with an excess of copper salt is free suboxide of copper formed. Creatinin interferes with TROMMER'S test for sugar, partly because it has a reducing action and partly by retaining the copper suboxide in solution. The combination with copper suboxide is not soluble in a saturated-soda solution, and if a little creatinin is dissolved in a cold, saturated-soda solution and then a few drops of FEHLING'S reagent added, a white flocculent combination separates after heating to 50–60° C. and then cooling (v. MASCHKE'S[1] reaction). An alkaline bismuth solution (see Sugar Tests) is not reduced by creatinin.

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[1] See Maly's Jahresber., Bd. 16, S. 199.

[2] Compt. rend. soc. biol., Tome 43. Cited from Maly's Jahresber., Bd. 21.

[3] This statement is taken from Huppert-Neubauer's book. Hoppe-Seyler's Handb., 6. Aufl., S. 144, gives other figures.

[4] Pflüger's Arch., Bd. 2, S. 220.

[5] Zeitschr. f. physiol. Chem., Bd. 5.

If we add a few drops of a freshly prepared very dilute sodium nitroprusside (sp. gr. 1.003) to a dilute creatinin solution (or to the urine) and then a few drops of caustic soda, a ruby-red liquid is obtained which quickly turns yellow again (WEYL'S[2] reaction). If we use ammonia instead of caustic soda in this reaction, the red color is not obtained (differing from acetone and diacetic acid, LE NOBEL[3]). If the above solution, which has become yellow, is treated with an excess of acetic acid and heated, the solution becomes first green and then blue (SALKOWSKI[4]); finally a precipitate of Prussian blue is obtained. If a solution of creatinin in water (or urine) is treated with a watery solution of picric acid and a few drops of a dilute caustic-soda solution, a red coloration lasting several hours occurs immediately at the ordinary temperature, and which turns yellow on the addition of acid (JAFFÉ'S[5] reaction). Acetone gives a more reddish-yellow color. Grape-sugar gives with this reagent a red coloration only after heating.

In preparing creatinin from urine the creatinin zinc chloride is first prepared according to NEUBAUER'S[6] method, and this method is also employed for the quantitative estimation of creatinin. In making a quantitative estimation 200–300 c. c. of urine freed from proteid (by boiling with acid) and from sugar (by fermentation with yeast) are measured, alkalized with milk of lime, and treated with $CaCl_2$ solution until all the phosphoric acid is precipitated; it is filtered and washed with water, the filtrate and the wash-water united, and evaporated to a syrup after acidifying with acetic acid. This syrup is mixed while hot with 50 c. c. of 95–97% alcohol. This mixture is transferred to a beaker, and the residue in the evaporating-dish is completely and carefully removed and added. The liquid is allowed to stand covered for at least eight hours in the cold. Then it is filtered through a small filter, the precipitate washed with alcohol, the filtrate evaporated if necessary until the volume is 50–60 c. c., then allowed to cool and ½ c. c. of an acid-free zinc-chloride solution of a specific gravity of 1.20 is added; it is stirred, and the covered beaker is left standing in a cool place for two or three days. The precipitate is collected on a small dried and weighed filter, using the filtrate to wash the crystals from the beaker. After allowing the crystals to completely drain off, they are washed with a little alcohol until the filtrate gives no reaction for chlorine, and dried at 100° C. 100 parts creatinin zinc-chloride contain 62.44 parts creatinin. As the precipitate is never quite pure, the quantity of zinc must be carefully determined, in exact experiments, by evaporating with nitric acid, heating, washing the oxide of zinc with water (to remove any NaCl), drying, heating, and weighing. 22.4 parts zinc oxide correspond to 100 parts creatinin zinc chloride.

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[1] Zeitschr. f. analyt. Chem., Bd. 17.

[2] Ber. d. deutsch. chem. Gesellsch., Bd. 11.

[3] Maly's Jahresber., Bd. 13, S. 238.

[4] Zeitschr. f. physiol. Chem., Bd. 4, S. 133.

[5] *Ibid.*, Bd. 10.

[6] Ann. d. Chem. u. Pharm., Bd. 119.

The preparation of creatinin zinc chloride on a large scale from urine is done in the same way. The creatinin is obtained from the creatinin zinc chloride by boiling with lead hydroxide, filtering, decolorizing the filtrate with animal charcoal, evaporating, treating the residue with strong alcohol (which leaves the creatin undissolved), evaporating to crystallization, redissolving in water, and recrystallizing.

In regard to the modifications of NEUBAUER's method for the quantitative estimation of creatinin the reader is referred to SALKOWSKI.[1] KOLISCH[2] has given a new method for estimating creatinin in urine which consists in precipitating the creatinin from the alcoholic extract by an alcoholic solution of mercuric chloride acidified with acetic acid. The nitrogen is exactly determined in the carefully washed precipitate by KJELDAHL's method. KOLISCH uses the following solution as precipitant: 30 parts mercuric chloride, 1 part sodium acetate, 3 drops glacial acetic acid, and 125 parts absolute alcohol.

Xanthocreatinin, $C_5H_{10}N_4O$. This body, which was first prepared fro meat extract by GAUTIER,[3] has been found by MONARI[4] in dog's urine afte the injection of creatinin into the abdominal cavity, and in human urine after several hours of exhausting marches. According to COLASANTI[1] it occurs to a relatively greater extent in lion's urine. STADTHAGEN[2] considers the xanthocreatinin, isolated from human urine after strenuous muscular activity, as impure creatinin.

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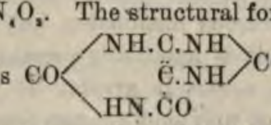
[1] Zeitschr. f. physiol Chem., Bdd. 10 and 14.

[2] Centralbl. f. innere Medizin, 1895.

[3] Bull. de l'Acad. de méd. (2), Tome 15, and Bull. de la soc. chim., Tom 48.

[4] See Maly's Jahresber., Bd. 17, S. 182.

Xanthocreatinin forms sulphur-yellow thin plates, similar to cholesterin, which have a bitter taste. It dissolves in cold water and in alcohol, and gives a crystalline combination with hydrochloric acid and a double compound with gold and platinum chloride. It gives a combination with zinc chloride, which crystallizes in fine needles. Xanthocreatinin has a poisonous action.

Uric Acid, $\overline{\mathrm{Ur}}$, $C_5H_4N_4O_3$. The structural formula of this acid, according to MEDICUS, is  $\mathrm{CO}\left\langle\begin{array}{l}\mathrm{NH.C.NH}\\ \qquad\ddot{\mathrm{C}}\mathrm{.NH}\\ \mathrm{HN.\dot{C}O}\end{array}\right.\!\!\begin{array}{l}\diagdown\\ \diagup\end{array}\!\mathrm{CO}$, and this acid may therefore be considered, from its constitution as a derivative of acrylic acid, as acrylic acid diureid.

Uric acid has been synthetically prepared by HORBACZEWSKI[3] in several ways. On fusing urea and glycocoll, uric acid is formed according to the formula $3CON_2H_4 + C_2H_5NO_2 = C_5H_4N_4O_3 + 2H_2O + 3NH_3$, and in this reaction hydantoin and biuret are formed as intermediate products. On melting methylhydantoin with urea or methylhydantoin with biuret or with allophanic-acid amyl-ester HORBACZEWSKI obtained methyl-uric acid. He also obtained uric acid on heating trichlor-lactic acid, or still better trichlor-lactic acid-amid, with an excess of urea. If we eliminate from the reaction the numerous by-products (cyanuric acid, carbon dioxide, etc.), then this process may be expressed by the formula $C_3Cl_3H_4O_2N + 2CON_2H_4 = C_5H_4N_4O_3 + H_2O + NH_4Cl + 2HCl$.

On strongly heating uric acid it decomposes with the formation of UREA, HYDROCYANIC ACID, CYANURIC ACID, and AMMONIA. On heating with concentrated hydrochloric acid in sealed tubes to 170° C. it splits into GLYCOCOLL, CARBON DIOXIDE, and AMMONIA. By the action of oxidizing agents a splitting and oxidation takes place, and either monoureid or diureid is produced. By oxidation with lead peroxide, CARBON DIOXIDE, OXALIC ACID, UREA, and ALLANTOIN, which last is glyoxyldiureid, are produced (see below).

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[1] Arch. ital. de Biologie, Tome 15.

[2] Zeitschr. f. klin. Med., Bd. 15.

[3] Monatshefte f. Chem., Bdd. 6 and 8. See also Behrend and Roosen, Ber. d. deutsch. chem. Gesellsch., Bd. 21, S. 999.

By oxidation with nitric acid in the cold UREA and a monoureid, the mesoxalyl urea or ALLOXAN, are obtained, $C_5H_4N_4O_3 + O + H_2O = C_4H_2N_2O_4 + (NH_2)_2CO$. On warming with nitric acid, alloxan yields carbon dioxide, and oxalyl urea or PARABANIC ACID, $C_3H_2N_2O_3$. By the addition of water the parabanic acid passes into OXALURIC ACID, $C_3H_4N_2O_4$, traces of which are found in the urine and which easily split into oxalic acid and urea.

Uric acid occurs most abundantly in the urine of birds and of scaly amphibians, in which animals the greater part of the nitrogen of the urine appears in this form. Uric acid occurs frequently in the urine of carnivorous mammalia, but is sometimes absent; in urine of herbivora it is habitually present, though only as traces; in human urine it occurs in greater but still small and variable amounts. Traces of uric acid are also found in several organs and tissues, as in the spleen, lungs, heart, pancreas, liver (especially in birds), and in the brain. It habitually occurs in the blood of birds (MEISSNER[1]). Traces have been found in human blood under normal conditions (ABELES[2]). Under pathological conditions it occurs to an increased extent in the blood in pneumonia (v. JAKSCH[3]), but also in leucamia and arthritis. Uric acid also occurs in large quantities in "chalk-stones," certain urinary calculi, and in guano. It has also been detected in the urine of insects and certain snails.

The amount of uric acid eliminated with the human urine is subject to considerable variation, but amounts on an average to 0.7 grm. during 24 hours on a mixed diet. The relationship of the uric acid to the urea on a mixed diet is on an average 1 : 50–1 : 70.[4] In new-born infants and in the first days of life the elimination of uric acid is increased (MAREŠ[5]), and the relation between the uric acid and urea is about 1 : 13–14. SJÖQVIST[6] found the relationship in new-born infants to be 1 : 6.42–17.1.

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[1] Zeitschr. f. rat. med. (3), Bd. 31. Cited from Hoppe-Seyler's Physiol. Chem., S. 432.

[2] Wien. med. Jahrbücher, 1887. Cited from Maly's Jahresber., Bd. 17.

[3] Ueber die klin. Bedeutung des Vorkommens der Harnsäure, etc. Prager Festschrift. Berlin, 1896. S. 79.

[4] A very good tabular summary of the variation in the elimination of uric acid and the relationship of total nitrogen to uric-acid nitrogen is found in v. Noorden's Lehrbuch der Pathologie des Stoffwechsels, S. 54.

[5] See Centralbl. f. d. med. Wissensch., 1888, S. 2.

[6] Nord. med. Arkiv., 1894, No. 10.

In regard to the action of food we know from the observations of RANKE,[1] MAREŠ,[2] and CAMERER[3] that the elimination of uric acid is diminished in starvation, and that it quickly increases on partaking food, especially proteid food. MAREŠ found the minimum about 13 hours after the last meal, and a strong increase about 2–5 hours after meat diet. This increase after a meal rich in proteid HORBACZEWSKI[4] explains by the digestion leucocytosis (see below) which habitually appears. It is quite generally accepted that the quantity of uric acid eliminated with vegetable food is smaller than with a meat diet, in which case the quantity may rise to 2 grms. or over per 24 hours.[5]

The statements in regard to the influence of other circumstances, as also of different bodies, on the elimination of uric acid are rather contradictory. This is in part due to the fact that the older investigators used an inaccurate method (HEINTZ'S method), and also, as shown especially by MAREŠ and SALKOWSKI,[6] that the extent of uric-acid elimination is dependent in the first place upon the individuality. According to SCHÖNDORFF[7] the drinking of water, contrary to older statements, does not have any effect on the elimination of uric acid. According to CLAR[8] and HAIG[9] alkalies increase the elimination of uric acid, while according to SALKOWSKI they diminish, and according to HERMANN[10] they have no influence on the elimination. HORBACZEWSKI and KANĚRA[11] found an increased elimination of uric acid after the administration of glycerin, while no increase was observed after partaking sodium acrylate (HORBACZEWSKI[12]). Certain medicines, such as quinin and atropin, diminish, while others, such as pilocarpin, increase, the elimination of uric acid. According to HORBACZEWSKI[13] and his pupils the first cause a diminution of the number of leucocytes in the blood, while the last cause an increase in the number.

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[1] J. Ranke, Beobachtungen und Versuche über die Ausscheidung der Harnsäure, etc. München, 1858.

[2] L. c.

[3] Zeitschr. f. Biologie, Bd. 26.

[4] Wien. Sitzungsber., Bd. 100, Abth. 3, 1891.

[5] In regard to the action of various diets the reader is referred to the above-cited authors, and especially to A. Hermann, Arch. f. klin. Med., Bd. 43.

[6] Virchow's Arch., Bd. 117.

[7] Pflüger's Arch., Bd. 46.

[8] Centralbl. f. d. med. Wissensch., 1888, No. 25.

[9] Journal of Physiol., Vol. 8.

[10] Arch. f. klin. Med., Bd. 43.

[11] Wien. Sitzungsber., Bd. 97.

[12] Monatshefte f. Chem., Bd. 10.

[13] Wien. Sitzungsber., Bd. 100.

Little is known in regard to the elimination of uric acid in disease. The uric acid introduced into the organism of a dog is in great part, as shown by FRERICHS and WÖHLER,[1] converted into urea, and as urea is also formed by the action of oxidizing agents on uric acid outside of the body, uric acid has been often considered as a step towards the formation of urea in the organism. Such a view is not, however, well founded, and the statement that in diseases with an incomplete supply of oxygen and diminished oxidation an increased formation of uric acid is produced has not been proved. With regard to the pathological relations we really only know two conditions in which the elimination of uric acid is increased, namely, in fever and leucæmia. In fevers the uric acid eliminated is increased after the crisis, but it is undecided whether the quantity is increased at the height of the fever as compared to the normal.[2] In leucæmia the elimination is increased absolutely as well as relatively to the urea (RANKE,[3] SALKOWSKI,[4] FLEISCHER and PENZOLDT,[5] STADTHAGEN,[6] STICKER,[7] BOHLAND and SCHURZ,[8] and others), and the relationship between the uric acid and urea (total nitrogen recalculated as urea) may be even 1 : 9, while under normal conditions, according to different investigators, it is 1 : 40 to 66 to 100. The elimination of uric acid may be diminished in gout shortly before and during the attack.

Formation of Uric Acid in the organism. The formation of uric acid in birds is increased by the administration of ammonia-salts (v. SCHRÖDER[9]). Urea acts in the same way (MEYER and JAFFÉ[10]), while in the organism of mammalia uric acid is more or less completely converted into urea. MINKOWSKI[11] observed in geese with extirpated livers a very significant decrease in the elimination of uric acid, while the elimination of ammonia was increased to a corresponding degree. This indicates a participation of ammonia in the formation of uric acid in the organism of birds; and as MINKOWSKI has also found after the extirpation of the liver that considerable amounts of lactic acid occur in the urine, it is probable that the uric acid in birds is produced in the liver, perhaps from lactic acid and ammonia by synthesis. Amido-acids—leucin, glycocoll, and aspartic acid—increase the elimination of uric acid in birds (v. KNIERIEM[1]), but whether the amido-acids are first decomposed with the splitting off of ammonia is still unknown. We have no basis for the statement as to the formation of uric acid from ammonium salts in the human and mammalian liver. v. MACH[2] has shown that a small part of the uric acid in birds originates from hypoxanthin, and a similar origin for the uric acid of mammalia is also very probable (MINKOWSKI).

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[1] Annal. d. Chem. u. Pharm., Bd. 65.

[2] See v. Noorden, Lehrbuch d. Pathol. des Stoffwechsels, S. 211 and 212.

[3] Schmidt's Jahrb., 1859.

[4] Virchow's Arch., Bd. 50.

[5] Arch. f. klin. Med., Bd. 26.

[6] Virchow's Arch., Bd. 109.

[7] Zeitschr. f. klin. Med., Bd. 14.

[8] Pflüger's Arch., Bd. 47.

[9] Zeitschr. f. physiol. Chem., Bd. 2.

[10] Ber. d. deutsch. Chem. Gesellsch., Bd. 10

[11] Arch. f. exp. Path. u. Pharm., Bd. 21.

The xanthin bases, as stated in Chapter V, originate from the nucleins, and HORBACZEWSKI[3] gives the same origin for uric acid. According to this investigator uric acid is not derived from the nuclein with the xanthin bases as intermediate steps, but uric acid or xanthin bases originate rather from the same mother-substance, the nuclein substances, according to circumstances.

Uric acid is formed when a cleavage precedes an oxidation, and xanthin bases, on the contrary, by cleavage without oxidation. Several circumstances speak for this origin of uric acid in the organism. HORBACZEWSKI has prepared uric acid from tissues rich in nuclein, such as the spleen-pulp, and from spleen nuclein by slight putrefaction, subsequent oxidation with blood, and then cleavage by boiling. If the oxidation was neglected, he obtained an equivalent quantity of xanthin bases. The nuclein prepared from the spleen-pulp when introduced into the animal body causes an increase in the elimination of uric acid, which HORBACZEWSKI considers is not due to a direct transformation of the nuclein. According to him it may be due indirectly to the leucocytosis produced by the nuclein. According to HORBACZEWSKI the uric acid originates chiefly from the nuclein of the destroyed leucocytes, and the greater the number of leucocytes in the blood the greater is the destruction of the same, and hence the elimination of uric acid is correspondingly increased. Observations on the elimination of uric acid stand in good accord with this theory. Thus, for example, new-born children eliminate more uric acid than adults because of the leucocytosis going on. The increase in the elimination of uric acid after food rich in proteid is explained by the leucocytosis, as also the abundant formation of uric acid, after animal as compared with vegetable food. Leucæmia, in which the elimination of uric acid is greatly increased, is characterized by an abnormally great number of leucocytes in the blood. Such medicaments, which increase the number of leucocytes, also increase in general[1] the elimination of uric acid.

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[1] Zeitschr. f. Biologie, Bd. 13.

[2] Arch. f. exp. Path. u. Pharm., Bd. 24.

[3] Wien Sitzungsber., Bd. 100.

It seems positively proven that a certain relationship exists between the elimination of uric acid and the quantity of leucocytes in the blood, and HORBACZEWSKI's view that the uric acid is a product of the destruction of the leucocytes is very acceptable. The positive proof that uric acid actually originates in the destruction of the leucocytes and not in some other way, in their reformation or as a metabolic product, has, as stated by MAREŠ,[2] not been given.

We cannot say anything positive in regard to the organ or organs in which uric acid is formed.

After the extirpation of the kidneys of snakes (ZALESKY[3]) and birds (V. SCHRÖDER[4]) an accumulation of uric acid in the blood and tissues has been observed. This shows that the kidneys of these animals are not the only organ producing uric acid, and any direct proof of the formation of this acid in the kidneys has not to the present time been demonstrated. A direct relationship between the spleen and the formation of uric acid, also in man, has been sought by several investigators. According to the investigations of HORBACZEWSKI this relationship seems to be of an indirect kind, as it probably stands in close connection with the importance of the spleen to the formation of the leucocytes. If uric acid is derived in man and mammals chiefly from nuclein, then we must look for its formation where a destruction of tissues containing nuclein takes place, although, according to HORBACZEWSKI, it originates in the first place in the destruction of the leucocytes. We have no positive basis for the statement that uric acid is formed in the liver of man and mammals, but the formation of uric acid in the liver of birds is shown to be highly probable by the researches of MINKOWSKI.

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[1] Horbaczewski, l. c.

[2] Wien Sitzungsber., Bd. 101, Abth. 3, and "Zur Theorie der Harnsäurebildung im Säugethierorganismus." Prag, 1892.

[3] Cited from Hermann's Handb., Bd. 5, Thl. 1, S. 305.

[4] Du Bois-Reymond's Arch., 1880, Suppl. Bd., and Ludwig's Festschrift. 1887.

Properties and Reactions of Uric Acid. Pure uric acid is a white, odorless, and tasteless powder consisting of very small rhombical prisms or plates. Impure uric acid is easily obtained as somewhat larger, colored crystals.

In quick crystallization, small, apparently colorless, thin, four-sided rhombic prisms are formed, which can only be seen by the aid of the microscope, and these sometimes appear as spools because of the rounding of their obtuse angles. The plates are sometimes six-sided, irregularly developed; in other cases they are rectangular with partly straight and partly jagged sides; and in other cases they show still more irregular forms, the so-called dumb-bells, etc. In slow crystallization, as when the urine deposits a sediment or when treated with acid, large, always colored crystals separate. Examined with the microscope these crystals appear always yellow or yellowish brown in color. The most ordinary form is the whetstone shape formed by the rounding off of the obtuse angles of the rhombic plate. The whetstones are generally connected together, two or more crossing each other. Besides these forms, rosettes of prismatic crystals, irregular crosses, brown-colored rough masses of destroyed needles and prisms occur, also other forms.

Uric acid is insoluble in alcohol and ether; it is rather easily soluble in boiling glycerin, very difficultly soluble in cold water (14,000–15,000 parts), and difficultly soluble in boiling water (in 1800–1900 parts). It is soluble in a warm solution of sodium diphosphate, and in the presence of an excess of uric acid monophosphate and acid urate are produced. Sodium phosphate is considered as a solvent for the uric acid in the urine. According to RÜDEL[1] urea is an important solvent. 1000 c. c. of a 2% urea solution can hold on an average 0.529 grm. uric acid in solution, and as the daily quantity of urine is 1500–2000 c. c., and this contains 2% urea, it is possible for the urea alone to hold nearly all of the uric acid eliminated in solution. Piperazin (diethylendiamin), $C_4H_{10}N_2$, is also a good solvent for uric acid. Uric acid dissolves without decomposing in concentrated sulphuric acid. It is completely precipitated from the urine by picric acid (JAFFÉ[1]).

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[1] Arch. f. exp. Path. u. Pharm., Bd. 30.

Uric acid is dibasic and correspondingly forms two series of salts, neutral and acid. According to BENCE JONES[2] hyperacid salts, QUADRIURATES, with the general formula ($MH\overline{U} + H_2\overline{U}$) also occur.

Of the alkali urates the neutral potassium and lithium salts dissolve most easily, and the ammonium salt dissolves with difficulty. The acid-alkali urates are very insoluble, and separate as a sediment (*sedimentum lateritium*) from concentrated urine on cooling. The salts with alkaline earths are very insoluble.

If a little uric acid in substance is treated on a porcelain dish with a few drops of nitric acid, the uric acid dissolves on warming with a strong development of gas, and after thoroughly drying on the water-bath a beautiful red residue is obtained, which turns a purple-red (ammonium purpurate or murexide) on the addition of a little ammonia. If, instead of the ammonia, we add a little caustic soda (after cooling), the color becomes more blue or bluish violet. This color disappears quickly on warming, differing from certain xanthin bodies. This reaction is called the *murexide test*.

If uric acid is converted into alloxan by the careful action of nitric acid and the excess of acid carefully expelled on treating this with a few drops concentrated sulphuric acid and commercial benzol (containing thiophen), a beautiful blue coloration is obtained (DENIGÈS'[3] reaction).

Uric acid does not reduce an alkaline solution of bismuth, but does, on the contrary, an alkaline copper-hydroxide solution. In the presence of only a little copper salt we obtain a white precipitate consisting of copper urate. In the presence of more copper salt red suboxide separates. The method for the volumetric estimation of uric acid as suggested by ARTHAUD and BUTTE,[4] as well as the method suggested by KRÜGER and WULFF,[5] is based on the insolubility of copper urate.

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[1] Zeitschr. f. physiol. Chem., Bd. 10.

[2] Journ. Chem. Soc., 1862, vol. xv., p. 8.

[3] Journal de Pharm. et de Chim., Tome 18. Cited from Maly's Jahresber., Bd. 18, S 24.

[4] Compt. rend. soc. biol., Tome 41. Cited from Maly's Jahresber., Bd. 20, S. 180

[5] Zeitschr. f. physiol. Chem., Bd. 20.

If a drop of uric acid dissolved in sodium carbonate is placed on a piece of filter-paper which has been previously treated with silver-nitrate solution, a reduction of silver oxide occurs producing a brownish-black or, in the presence of only 0.002 milligramme uric acid, a yellow spot (SCHIFF'S[1] test).

Preparation of Uric Acid from Urine. Filtered normal urine is treated with 20–30 c. c. of 25% hydrochloric acid for each litre of urine. After forty-eight hours collect the crystals and purify them by redissolving in dilute alkali, decolorizing with animal charcoal and reprecipitating with hydrochloric acid. Large quantities of uric acid are easily obtained from the excrements of serpents by boiling them with dilute caustic potash until no more ammonia is developed. A current of carbon dioxide is passed through the filtrate until it barely has an alkaline reaction; dissolve the separated and washed acid potassium urate in caustic potash, and precipitate the uric acid by addition of an excess of hydrochloric acid to the filtrate.

Quantitative Estimation of Uric Acid in the urine. As the older method as suggested by HEINTZ, even after recent modifications, gives inaccurate results, we will not give it in detail.

SALKOWSKI[2] and LUDWIG'S[3] method consists in precipitating by silver nitrate the uric acid from the urine previously treated with magnesia-mixture, and weighing the uric acid obtained from the silver precipitate. Uric-acid determinations by this method are often performed according to the suggestion of E. LUDWIG, which requires the following solutions:

1. An AMMONIACAL SILVER-NITRATE solution, which contains in one litre 26 grms. silver nitrate and a quantity of ammonia sufficient to completely redissolve the precipitate produced by the first addition of ammonia. 2. MAGNESIA MIXTURE. Dissolve 100 grms. crystallized magnesium chloride in water and add enough ammonia so that the liquid smells strongly of it, and enough ammonium chloride to dissolve the precipitate and dilute to 1 litre. 3. SODIUM-SULPHIDE SOLUTION. Dissolve 10 grms. caustic soda which is free from nitric acid and nitrous acid in 1 litre of water. One half of this solution is completely saturated with sulphuretted hydrogen and then mixed with the other half.

The concentration of the three solutions is so arranged that 10 c. c. of each is sufficient for 100 c. c. of the urine.

100–200 c. c., according to concentration, of the filtered urine freed from proteid (by boiling after the addition of a few drops of acetic acid) are poured into a beaker. In another vessel mix 10–20 c. c. of the silver solution with 10–20 c. c. of the magnesia mixture and add ammonia, and when necessary also some ammonium chloride, until the mixture is clear. This solution is added to the urine while stirring, and the mixture allowed to stand quietly for half an hour. The precipitate is collected on a filter, washed with ammoniacal water, and then returned to the same beaker by the aid of a glass rod and a spirt-bottle, without destroying the filter. Now heat to boiling 10–20 c. c. of the alkali-sulphide solution, which has previously been diluted with an equal volume of water, and allow this solution to flow through the above filter into the beaker containing the silver precipitate, wash with boiling water, and warm the contents of the beaker on a water-bath for a time, stirring constantly. After cooling filter into a porcelain dish, wash with boiling water, acidify the filtrate with hydrochloric acid, evaporate to about 15 c. c., add a few drops more of hydrochloric acid, and allow it to stand for 24 hours. The uric acid which has crystallized is collected on a small weighed filter, washed with water, alcohol, ether, and carbon disulphide, dried at 100–110° C. and weighed. For each 10 c. c. of watery filtrate we must add 0.00048 grm. uric acid to the quantity found directly. Instead of the weighed filter-paper a glass tube filled with glass-wool as described in other handbooks may be substituted (Ludwig). Too strong or continuous heating with the alkali sulphide must be prevented, otherwise a part of the uric acid may be decomposed. Grove[1] recommends a solution of potassium iodide instead of the alkali sulphide, thus making the washing with carbon disulphide unnecessary. Camerer[2] has modified this method in certain points, and he determines the nitrogen in the silver precipitate (a-uric acid = uric acid contaminated with xanthin bodies) and also the uric acid isolated by Salkowski-Ludwig's method (= b-uric acid).

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[1] Annal. d Chem. u. Pharm., Bd. 109.

[2] Virchow's Arch., Bd. 52, and Pflüger's Arch., Bd. 5.

[3] Wien. med. Jahrb., 1884, and Zeitschr. f. analyt. Chem., Bd. 24.

Haycraft's Method.[3] 25 c. c. of the urine are first treated with 1 grm. bicarbonate, then made strongly alkaline by ammonia, and lastly precipitated by an ammoniacal silver solution. The carefully washed precipitate is dissolved in 20–30% nitric acid and this solution titrated with a $\frac{n}{100}$ sulphocyanide solution according to Volhard's method. Each c. c. of this solution corresponds to 0.00168 grm. uric acid. This method has been modified in certain points by Hermann[4] and Czapek,[5] which last titrates with alkali sulphide the silver salts remaining in solution in the urine after the precipitation of the uric acid by a known volume of ammoniacal silver solution of known strength. The advantage of Haycraft's method is the ease and rapidity with which it can be performed, and it is therefore recommended for clinical purposes. For exact determinations it is not quite reliable, because the amount of silver in the precipitate of silver urate is not constant (SALKOWSKI[1]). HAYCRAFT'S method gives the same results as SALKOWSKI-LUDWIG'S method in pure uric-acid solutions. With the urine HAYCRAFT'S method gives on the contrary too high results, which is in part due to the fact that the silver solution precipitates from the urine other bodies, such as xanthin bases, besides the uric acid. Since the value of this method has been the subject of much adverse criticism, we will not give further particulars.[2]

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[1] Journ. of Physiol., Bd. 12.

[2] Zeitschr. f. Biologie, Bdd. 27 u. 28.

[3] Zeitschr. f analyt. Chem., Bd. 25.

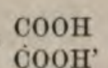
[4] Zeitschr. f. physiol. Chem., Bd. 12.

[5] *Ibid.*, Bd. 12, S. 502.

In regard to FOKKER'S[3] method we refer the reader to more exhaustive text-books.

HOPKINS'S[4] method is based on the fact that the uric acid is completely precipitated from the urine as ammonium urate on saturating with ammonium chloride. The urine is saturated with ammonium chloride (for each 100 c. c. urine add 30 grms. ammonium chloride) and filter after two hours. Wash with a saturated solution of ammonium chloride, and transfer the precipitate from filter to a small beaker by means of boiling water, and decompose it with hydrochloric acid and heat. The uric acid which separates is determined by weighing it as such, or by titration with potassium permanganate. This simple method gives as good results as SALKOWSKI-LUDWIG'S method. KRÜGER and WULFF'S method will be treated of in connection with xanthin bases in the urine.

OXALURIC ACID, $C_3H_4N_2O_4 = (CON_2H_3)\,CO.COOH$. This acid, whose relation to uric acid and urea has been spoken of above, occurs only as traces in the urine as ammonium salts. This salt is not directly precipitated by $CaCl_2$ and NH_3, but after boiling, when it is decomposed into urea and oxalate. In preparing oxaluric acid from urine the latter is filtered through animal charcoal. The oxalurate retained by the charcoal may be obtained by boiling with alcohol.

Oxalic Acid, $C_2H_2O_4$, or  $\begin{array}{c}COOH\\ \dot{C}OOH\end{array}$, occurs under physiological conditions in very small amounts in the urine, about 0.02 grm. in 24 hours (FÜRBRINGER[5]). According to the generally accepted view it exists in the urine as calcium oxalate, which is kept in solution by the acid phosphates present. Calcium oxalate is a frequent constituent of urinary sediments, and occurs also in certain urinary calculi.

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[1] Pflüger's Arch., Bd. 5; also Salskowski and Jolin, Zeitschr. f. physiol. Chem., Bd. 14.

[2] In regard to the literature on this subject see Huppert-Neubauer's Harnanalyse. See also Lisowski, Maly's Jahresber., Bd. 20; Deroide, *ibid.*, Bd. 21, S. 172; Groves, l. c., and Haycraft, Zeitschr. f. physiol. Chem., Bd. 15.

[3] Pflüger's Arch., Bd. 10.

[4] Journal of Pathology and Bacteriology, 1893, and Proceedings of Royal Society, Vol. 52.

[5] Deutsch. Arch. f. klin. Med., Bd. 18.

The origin of the oxalic acid in the urine is not well known. Oxalic acid when administered is eliminated, at least in part, by the urine unchanged, and as many vegetables and fruits, such as cabbage, spinach, asparagus, sorrel, apples, grapes, etc., contain oxalic acid, it is possible that a part of the oxalic acid of the urine originates directly from the food. According to Abeles[1] this is not the case. According to him an alimentary oxaluria, that is, an elimination of oxalic acid caused by partaking of the ordinary foods containing oxalic acid, does not exist, and the soluble oxalates of the food are in all probability converted into insoluble lime-salts in the digestive tract. That oxalic acid may be formed in the animal body from proteid or fat follows from the observations of Mills[2] that oxalic acid is found in the urine of dogs after feeding with meat and fat alone. Oxalic acid is also supposed to be derived by the incomplete combustion of carbohydrates, and is also considered, but not with sufficient basis, as an oxidation product of uric acid.

An increased elimination of oxalic acid may occur in diabetes. The question whether it occurs as an independent disease (*oxaluria*, oxalic-acid diathesis) has not been positively decided.

The properties and reactions of oxalic acid and calcium oxalate are well known. Calcium oxalate as a constituent of urinary sediments will be described later.

Detection and Quantitative Estimation of Oxalic Acid in Urine. The presence of oxalic acid in solution in urine is determined according to the method suggested by Neubauer,[3] who treats 500–600 c. c. of the urine with $CaCl_2$ solution, makes alkaline with ammonia and then faintly acid with acetic acid. After 24 hours the precipitate is collected on a small filter, washed with water, treated with hydrochloric acid (which leaves the uric acid undissolved on the filter), and washed again with water. The filtrate, including the wash-water, is treated with an excess of ammonia and allowed to stand 24 hours. Calcium oxalate separates as quadratic octahedra. The quantitative estimation is performed after the same principle. The oxalate is converted into quicklime by heat, and weighed as such.

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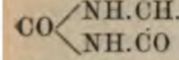
[1] Wien. klin. Wochenschr., 1892.

[2] Virchow's Arch., Bd. 91.

[3] Zeitschr. f. analyt. Chem., Bd. 8, S. 521.

Allantoin or GLYOXYLDIUREID, $C_4H_6N_4O_3$ or



$CO\left\langle\begin{matrix}NH.CH.NH.CO.NH_2\\NH.CO\end{matrix}\right.$, occurs in the urine of children within the first eight days after birth, and in very small amounts also in the urine of adults (Gusserow,[1] Ziegler and Hermann[2]). It is found in rather abundant quantities in the urine of pregnant women (Gusserow). Allantoin has also been found in the urine of sucking calves (Wöhler[3]), and sometimes in the urine of other animals (Meissner[4]). It is also found in the amniotic fluid and, as first shown by Vauquelin[5] and Lassaigne,[6] in the allantoic fluid of the cow (hence the name). Allantoin is formed, as above stated, by the oxidation of uric acid. The increased elimination of allantoin which Salkowski[7] observed in dogs after the administration of uric acid shows that the formation of allantoin from uric acid in the organism is not improbable. Borissow[8] has observed an abundant elimination of allantoin in dogs after poisoning with diamid.

Allantoin is a colorless substance often crystallizing in prisms, difficultly soluble in cold water, easily soluble in boiling water and also in warm alcohol, but not soluble in cold alcohol or ether. It combines with acids, forming salts. A watery allantoin solution gives no precipitate with silver nitrate alone, but by the careful addition of ammonia a white flocculent precipitate is formed, $C_4H_5AgN_4O_3$, which is soluble in an excess of ammonia and which consists after a certain time of very small, transparent microscopic globules. The dried precipitate contains 40.75% silver. A watery allantoin solution is precipitated by mercuric nitrate. On continuous boiling allantoin reduces Fehling's solution. It gives Schiff's furfurol reaction less rapidly and less intensely than urea. Allantoin does not give the murexid test.

Allantoin is most easily prepared by the oxidation of uric acid with lead peroxide. In preparing allantoin from calves' urine, concentrate the urine on the water-bath to a syrup and allow it to stand in the cold for several days. The crystals which are separated from the precipitate by washing are dissolved in boiling water with the addition of some animal charcoal, and filtered while hot; then acidify the filtrate faintly with hydrochloric acid (so as to keep the phosphates in solution) and allow it to crystallize. Allantoin is detected in human urine by the method first suggested by MEISSNER.[1] It consists chiefly of the following points: Precipitate the urine with baryta-water, filter, remove the baryta with sulphuric acid, filter, precipitate the allantoin with $HgCl_2$ in alkaline solution, decompose the precipitate with sulphuretted hydrogen, concentrate strongly, purify the crystals which separate by recrystallization, and lastly prepare the silver combination.

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[1] Arch. f. Gynäkol., Bd. 3.

[2] See Gusserow, *ibid.*

[3] Nachr. d. k. Gesellsch. d. Wissensch. zu Göttingen, 1849. Cited from Hoppe-Seyler's Physiol. Chem., S. 816.

[4] Zeitschr. f. rat. Med. (3), Bd. 31.

[5] Annal. d. Chem., Bd. 33.

[6] Annal. de chim. et de phys., Tome 17.

[7] Ber. d. deutsch. chem. Gesellsch., Bd. 9.

[8] Zeitschr. f. physiol. Chem., Bd. 19.

Xanthin Bases. The xanthin bases which habitually occur in human urine are *xanthin*, *hypoxanthin* (SALOMON[2]), *guanin* (POUCHET[3]), *carnin* (POUCHET), *paraxanthin* (THUDICHUM,[4] SALOMON[5]), *heteroxanthin* (SALOMON[6]), and *episarkin* (BALKE[7]). The quantity of these bodies in the urine is very small. The quantity of xanthin bodies in the urine is increased especially in leucæmia, in which disease *adenin* is also found in the urine (STADTHAGEN[8]). An increased elimination of certain xanthin bases has also been observed by POUCHET in fevers and affections of the nervous system. KRUGER[9] has found two new xanthin bases in the urine of lunatics. One of these, *epiguanin*, is similar to guanin in solubilities and has the formula $C_{10}H_{13}N_9O_2$. The second could not be obtained in sufficient quantity for analysis. Xanthin also occurs as a constituent of a variety of rare calculi (MARCET). It is also sometimes found as a constituent of urinary sediments (BENCE JONES).

Paraxanthin, $C_7H_8N_4O_2$ (dimethylxanthin), and **heteroxanthin**, $C_6H_6N_4O_2$ (methylxanthin), do not give the xanthin reaction with nitric acid and alkali, but give WEIDEL'S reaction (see page 105). They differ from other xanthin bodies by forming crystalline combinations with alkalies which are difficultly soluble. Amorphous heteroxanthin separates on neutralizing the sodium combination, but paraxanthin, on the contrary, separates in a crystalline condition. Paraxanthin gives an easily soluble combination with hydrochloric acid, while heteroxanthin forms an insoluble, beautiful crystalline combination.

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[1] Zeitschr. f. rat. Med., Bd. 31.

[2] Reichert's and Du Bois-Reymond's Arch., 1876; Du Bois-Reymond's Arch., 1882; and Zeitschr. f. physiol. Chem., Bd. 11.

[3] Contributions à la connaissance des matières extractives de l'urine. Thèse. Paris, 1880. Cited from Huppert-Neubauer.

[4] Gründzüge d. anat. und klin. Chem. Berlin, 1886.

[5] Du Bois-Reymond's Arch., 1882; Ber. d. deutsch. chem. Gesellsch., Bdd. 16 and 18.

[6] Du Bois-Reymond's Arch., 1885; Ber. d. deutsch. chem. Gesellsch., Bd. 18; and Zeitschr. f. physiol. Chem., Bd. 11.

[7] Zur Kenntniss der Xanthinkörper. Inaug.-Diss. Leipzig, 1893.

[8] Virchow's Arch., Bd. 109.

[9] Du Bois-Reymond's Arch., 1894.

Episarkin is the name given by BALKE to a new xanthin base occurring in human urine. The same body has been observed by SALOMON[1] in pig's and dog's urine, as well as in urine in leucæmia. BALKE gives $C_4H_6N_3O$ as the probable formula for episarkin. It is nearly insoluble in cold water, dissolves with difficulty in hot water, but may be obtained therefrom as long fine needles. Episarkin does not give the xanthin reaction with nitric acid nor WEIDEL'S reaction. It gives, on the contrary, the murexid test with hydrochloric acid and potassium chlorate. The silver combination is difficultly soluble in nitric acid.

In preparing xanthin bodies from the urine, it is supersaturated with ammonia and precipitated by a silver-nitrate solution. The precipitate is then decomposed with sulphuretted hydrogen. The boiling-hot filtrate is evaporated to dryness and the dried residue treated with 3% sulphuric acid. The xanthin bodies are dissolved, while the uric acid remains undissolved. This filtrate is saturated with ammonia and precipitated by silver-nitrate solution. The different xanthin bodies may be separated from each other by treating the silver precipitate with boiling-hot nitric acid of a sp. gr. of 1.1 (see page 108).

The xanthin bases may be quantitatively estimated according to the following method as suggested by KRÜGER and WULFF.[2] This method is based on the property of the xanthin bases and uric acid of being completely precipitated as an insoluble copper-oxide combination on the addition of copper-sulphate and sodium-bisulphite solution.

The author uses a 13% copper-sulphate solution, a 50% bisulphite solution, and a 10% $BaCl_2$ solution. The addition of $BaCl_2$ has the purpose of facilitating the settling and filtration of the precipitated copper oxide combination by the $BaSO_4$ formed. 100 c. c. of the urine, free from proteid, is heated to boiling, 10 c. c of the bisulphite solution added and immediately thereupon 10 c. c. of the copper-sulphate solution, and again heated to boiling. Then add 5 c. c. $BaCl_2$ solution and allow to settle for two hours, filter and wash completely with boiled water which has been cooled to 60° C. The filter and contents are treated according to KJELDAHL-GUNNING and the nitrogen determined. This nitrogen is the total nitrogen of the uric acid and xanthin bases. If the nitrogen of the uric acid precipitated according to SALKOWSKI-LUDWIG'S method is determined in another portion of the urine, then the difference between these two results gives the nitrogen of the xanthin bases. This multiplied by 2.755 gives the total quantity of xanthin bases.

If uric acid is to be determined by this method, the copper-oxide precipitate is treated with sodium sulphide, filtered, acidified with hydrochloric acid, concentrated by evaporation, and the precipitated uric acid collected on a filter after a certain time. We refer the reader to the original article in regard to the method of estimating uric acid and xanthin bases as suggested by SALKOWSKI.[3]

Hippuric acid, or BENZOYL-AMIDO-ACETIC ACID, $C_9H_9NO_3$ or $C_6H_5.CO.NH.CH_2.COOH$. This acid decomposes into benzoic acid and glycocoll on boiling the urine with mineral acids or alkalies, and also by putrefaction. The reverse of this occurs if these two components are heated in a sealed tube according to the following equation: $C_6H_5COOH + NH_2.CH_2.COOH = C_6H_5.CO.NH.CH_2.COOH + H_2O$. This acid may be synthetically prepared from benzamid and monochlor-acetic acid, $C_6H_5.CO.NH_2 + CH_2Cl.COOH = C_6H_5.CO.NH.CH_2.COOH + HCl$, also in other different ways.

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[1] Zeitschr. f. physiol. Chem., Bd. 18.

[2] *Ibid*., Bd. 20.

[3] Centralbl. f. d. med. Wissensch., 1894, No. 30.

Hippuric acid occurs in large amounts in the urine of herbivora, but only in small quantities in that of carnivora. The quantity of hippuric acid eliminated in human urine on a mixed diet is usually less than 1 grm. per 24 hours; as an average it is 0.7 grm. After eating freely of vegetables and fruit, especially such fruit as plums, the quantity may be more than 2 grms. Hippuric acid is also found in the perspiration, blood, suprarenal capsule of oxen, and in ichthyosis scales. Nothing is positively known in regard to the quantity of hippuric acid in the urine in disease.

The *Formation of Hippuric Acid* in the organism. Benzoic acid and also the substituted benzoic acids are converted into hippuric acid and substituted hippuric acids within the body. Also, those bodies are transformed into hippuric acid which by oxidation (toluol, cinnamic acid, hydrocinnamic acid) or by reduction (quinic acid) are converted into benzoic acid. The question of the origin of hippuric acid is therefore connected with the question of the origin of benzoic acid; for the formation of the second component, glycocoll, from the protein substances in the body is without question.

Hippuric acid is found in the urine of starving dogs (SALKOWSKI[1]), also in dog's urine after a diet consisting entirely of meat (MEISSNER and SHEPARD,[2] SALKOWSKI, and others). It is evident that the benzoic acid originates in these cases from the proteids. Benzoic acid may indeed be produced outside of the body by the oxidation of proteids; the benzoic acid produced on a diet consisting entirely of meat seems to be derived from the putrefaction of the proteids in the intestine. Among the products of the putrefaction of proteid outside of the body SALKOWSKI[3] has found phenylpropionic acid, $C_6H_5.CH_2.CH_2.COOH$, which is oxidized in the organism to benzoic acid and eliminated as hippuric acid after combining with glycocoll. Phenylpropionic acid seems to be formed from the amidophenylpropionic acid, which is prepared only from the plant proteids, and the supposition that the phenylpropionic acid is produced from tyrosin by putrefaction in the intestine has not been substantiated by the researches of BAUMANN,[1] SCHOTTEN,[2] and BAAS.[3] The importance of putrefaction in the intestine in producing hippuric acid is evident from the fact that after thoroughly disinfecting the intestine of dogs with calomel the hippuric acid disappears from the urine (BAUMANN[4]).

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[1] Ber. d. deutsch. chem. Gesellsch., Bd. 11.

[2] Untersuch. über das Entstehen der Hippursäure im thierischen Organismus. Hannover, 1866

[3] E. and H. Salkowski, Ber. d. deutsch chem. Gesellsch., Bd. 12.

The large quantity of hippuric acid present in the urine of herbivora is partly explained by the fact that vegetable proteids yield perhaps larger amounts of amidophenylpropionic acid, and partly by the specially active processes of putrefaction going on in the intestine of herbivora. These circumstances do not entirely explain this excess of hippuric acid. The abundant elimination of hippuric acid by herbivora may in part depend on the great amount of aromatic substances in the food of these animals which is converted into benzoic acid in the organism. There is hardly any doubt that the hippuric acid in human urine after a mixed diet, and especially after a diet of vegetables and fruits, has in part a similar origin.

The kidneys may be considered in dogs as special organs for the synthesis of hippuric acid (SCHMIEDEBERG and BUNGE[5]). In other animals, as in rabbits, the formation of hippuric acid seems to take place in other organs, such as the liver and muscles. The synthesis of hippuric acid is therefore not exclusively limited to any special organ, though perhaps in some species of animals it may be more abundant in one organ than in another.

Properties and *reactions* of hippuric acid. This acid crystallizes in semi-transparent, milk-white, long, four-sided rhombic prisms or columns, or in needles by rapid crystallization. They dissolve in 600 parts cold water, but more easily in hot water. They are easily soluble in alcohol, but with difficulty in ether. They are more easily soluble (about 12 times) in acetic ether than in ethyl ether. Petroleum ether does not dissolve them.

On heating hippuric acid it first melts at 187.5° C. to an oily liquid which crystallizes on cooling. By continuing the heat it decomposes, producing a red mass and a sublimate of benzoic acid, with the generation, first, of a peculiar pleasant odor of hay, and then an odor of hydrocyanic acid. Hippuric acid is easily differentiated from benzoic acid by this behavior, also by its crystalline form and its insolubility in petroleum ether. Hippuric acid and benzoic acid both give LÜCKE's reaction, namely, they generate an intense odor of nitrobenzol when evaporated with nitric acid to dryness and when the residue is heated. Hippuric acid forms crystallizable salts, in most cases, with bases. The combinations with alkalies and alkaline earths are soluble in water and alcohol. The silver, copper, and lead salts are soluble with difficulty in water; the iron-oxide salt is insoluble.

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[1] Zeitschr. f. physiol. Chem., Bd. 7.

[2] *Ibid.*, Bd. 8.

[3] *Ibid.*, Bd. 11.

[4] *Ibid.*, Bd. 10, S. 131.

[5] Arch. f. exp. Path. u. Pharm., Bd. 6. Also Ar. Hoffmann, *ibid.*, Bd. 7, and Kochs, Pflüger's Arch., Bd. 20.

Hippuric acid is best prepared from the fresh urine of a horse or cow. The urine is boiled a few minutes with an excess of milk of lime. The liquid is filtered while hot, concentrated and then cooled, and the hippuric acid precipitated by the addition of an excess of hydrochloric acid. The crystals are pressed, dissolved in milk of lime by boiling, and treated as above; the hippuric acid is precipitated again from the concentrated filtrate by hydrochloric acid. The crystals are purified by recrystallization and decolorized, when necessary, by animal charcoal.

The quantitative estimation of hippuric acid in the urine may be performed by the following method (BUNGE and SCHMIEDEBERG[1]): The urine is first made faintly alkaline with soda, evaporated nearly to dryness, and the residue thoroughly extracted with strong alcohol. After the evaporation of the alcohol dissolve in water, acidify with sulphuric acid, and completely extract by agitating (at least five times) with fresh portions of acetic ether. The acetic ether is then repeatedly washed with water, which is removed by means of a separatory funnel, then evaporated at a medium temperature, and the dry residue treated repeatedly with petroleum ether, which dissolves the benzoic acid, oxyacids, fat, and phenol, while the hippuric acid remains undissolved. This residue is now dissolved in a little warm water and evaporated at 50–60° C. to crystallization. The crystals are collected on a small weighed filter. The mother-liquor is repeatedly shaken with acetic ether. This last is removed and evaporated; the residue is added to the above crystals on the filter, dried and weighed.

Phenaceturic Acid, $C_{10}H_{11}NO_3 = C_6H_5.CH_2.CO.NH.CH_2.COOH$. This acid, which is produced in the animal body by a grouping of the phenylacetic acid, $C_6H_5.CH_2.COOH$, formed by the putrefaction of the proteids with glycocoll, has been prepared from horse's urine by SALKOWSKI,[2] but it probably also occurs in human urine.

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[1] L. c.

[2] Zeitschr. f. physiol. Chem., Bd. 9. See also E. and H. Salkowski, *ibid.*, Bd. 7.

Benzoic Acid, $C_7H_6O_2$ or $C_6H_5.COOH$, is found in rabbit's urine and sometimes, though in small amounts, in dog's urine (Weyl and v. Anrep[1]). According to Jaarsveld and Stokvis[2] and to Kronecker[3] it is also found in human urine in diseases of the kidneys. The occurrence of benzoic acid in the urine seems to be due to a fermentative decomposition of hippuric acid. Such a decomposition may very easily occur in an alkaline urine or one containing proteid (Van de Velde and Stokvis[4]). In certain animals—pigs and dogs—the kidneys, according to Schmiedeberg[5] and Minkowski,[6] contain a special enzyme, Schmiedeberg's *histozym*, which splits the hippuric acid with the separation of benzoic acid.

Ethereal Sulphuric Acids. In the putrefaction of proteids in the intestine, phenol, whose mother-substance is considered to be tyrosin, and indol and skatol are produced. The two last-named bodies, after they have been oxidized into indoxyl and skatoxyl, pass into the urine as ethereal sulphuric acids after uniting with sulphuric acid. The most important of these ethereal acids are *phenol-* and *cresol-sulphuric acid*—which were formerly also called phenol-forming substance—*indoxyl-* and *skatoxyl-sulphuric acid.* To this group belong also the *pyrocatechin-sulphuric acid*, which only occurs in very small amounts in human urine, and *hydrochinon-sulphuric acid*, which appears in the urine after poisoning with phenol, and perhaps under physiological conditions other ethereal acids occur which have not been isolated. The ethereal sulphuric acids of the urine were discovered and specially studied by Baumann.[7] The quantity of these acids in human urine is small, while horse's urine contains larger quantities. According to the determinations of v. d. Velden[8] the quantity of ethereal sulphuric acid in human urine in the 24 hours varies between 0.094 and 0.620 grms. The relationship of the sulphate-sulphuric acid *A* to the conjugated sulphuric acid *B* in health is on an average as 10 : 1. It undergoes such great variation, as found by Baumann and Herter[9] and after them by many other investigators, that it is hardly possible to consider the average figures as normal. After taking phenol and certain other aromatic substances, as also with abundant putrefaction within the organism, the elimination of ethereal sulphuric acid is greatly increased. On the contrary it is diminished when the putrefaction in the intestine is reduced or prevented. For this reason it may be greatly diminished by carbohydrates and one-sided milk diet.[1] The elimination of ethereal sulphuric acid has also been diminished in certain cases by certain therapeutic agents which have an antiseptic acid; still the statements are not unanimous.[2]

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[1] Zeitschr. f. physiol. Chem., Bd. 4.

[2] Arch. f. exp. Path. u. Pharm., Bd. 10.

[3] *Ibid.*, Bd. 16.

[4] *Ibid.*, Bd. 17.

[5] *Ibid.*, Bd. 14, S. 379.

[6] *Ibid.*, Bd. 17.

[7] Pflüger's Arch., Bdd. 12 and 13.

[8] Virchow's Arch., Bd. 70.

[9] Zeitschr. f. physiol. Chem., Bd. 1.

Great weight has been put upon the relationship between the total sulphuric acid and the conjugated sulphuric acid, or between the conjugated sulphuric acid and the sulphate-sulphuric acid, in the study of the intensity of the putrefaction in the intestine under different conditions. Several investigators, F. Müller,[3] Salkowski,[4] and v. Noorden,[5] consider correctly that this relationship is only of secondary value and that it is more correct to consider the absolute value. It must be remarked that the absolute values for the conjugated sulphuric acid also undergo great variation, so that it is at present impossible to give the upper or lower limit for the normal value.

Phenol- and p-Cresol-sulphuric Acid, $C_6H_5.O.SO_2.OH$ and $C_7H_7.O.SO_2.OH$. These acids are found as alkali salts in human urine, in which also orthocresol has been detected. The quantity of cresol-sulphuric acid is considerably greater than phenol-sulphuric acid. In the quantitative estimation the phenols set free from the two ethereal acids are determined together as tribromphenol. The quantity of phenols which are separated from the ethereal sulphuric acids of the urine amounts to 17–51 milligrammes in the 24 hours (Munk[6]). The methods for the quantitative estimation used heretofore give, according to Rumpf[7] and also Kossler and Penny,[8] such inaccurate results that new determinations are very desirable. After a vegetable diet the quantity of these ethereal-sulphuric acids is greater than after a mixed diet. After taking carbolic acid, which is in great part converted by synthesis within the organism into phenol-ethereal-sulphuric acid, besides also pyrocatechin- and hydrochinon-sulphuric acid,[1] and also when the amount of sulphuric acid is not sufficient to combine with the phenol, forming phenyl-glycuronic acid,[2] the quantity of phenols and ethereal-sulphuric acids in the urine is considerably increased at the expense of the sulphate-sulphuric acid.

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[1] See Hirschler, Zeitschr. f. physiol. Chem., Bd. 10; Biernacki, Deutsch. Arch. f. klin. Med., Bd. 49; Rovighi, Zeitschr. f. physiol. Chem., Bd. 16; Winternitz, *ibid.*, and Schmitz, *ibid.*, Bdd. 17 and 19.

[2] See Baumann and Morax, Zeitschr. f. physiol. Chem., Bd. 10; Steiff, Zeitschr. f. klin. Med., Bd. 16; Rovighi, l. c.; Stern, Zeitschr. f. Hygiene, Bd. 12; and Bartoschewitsch, Zeitschr. f. physiol. Chem., Bd. 17.

[3] Zeitschr. f. klin. Med., Bd. 12.

[4] Zeitschr. f. physiol. Chem., Bd. 12.

[5] Zeitschr. f. klin. Med., Bd. 17.

[6] Pflüger's Arch., Bd. 12.

[7] Zeitschr. f. physiol. Chem., Bd. 16.

[8] *Ibid.*, Bd. 17.

An increased elimination of phenol-sulphuric acids occurs in active putrefaction in the intestine with stoppage of the contents of the intestine, as in ileus, diffused peritonitis with atony of the intestine, or tuberculous enteritis, but not in simple obstruction. The elimination is also increased by the absorption of the products of putrefaction from purulent wounds or abscesses. An increased elimination of phenol has been observed in a few other cases of diseased conditions of the body.[3]

The alkali salts of phenol- and cresol-sulphuric acids crystallize in white plates, similar to mother-of-pearl, which are rather freely soluble in water. They are soluble in boiling alcohol, but only slightly soluble in cold. On boiling with dilute mineral acids they are decomposed into sulphuric acid and the corresponding phenol.

Phenol-sulphuric acids have been synthetically prepared by BAUMANN from potassium pyrosulphate and phenol- or p-cresol-potassium. For the method of their preparation from urine, which is rather complicated, the reader is referred to other text-books. The quantitative estimation of these ethereal sulphuric acids is done by determining the amount of phenol which may be separated from the urine as tribromphenol. In this determination, when the urine is not specially rich in phenol, about one fourth of the total quantity in the 24 hours is used; it is acidified with concentrated hydrochloric acid—5 c. c. for every 100 c. c. of urine—and distilled until a portion of the distillate does not give the slightest reaction for phenol with MILLON's reagent or with bromine-water. The distillate is now carefully neutralized with soda solution (which combines with the benzoic acid, etc.) and again distilled until a portion of the distillate is free from phenol, as shown by the above-mentioned reagents. This distillate is treated with bromine-water until a permanent yellow color is produced, and then allowed to stand for about 24 hours in the cold; the crystalline precipitate is then collected on a small weighed filter, washed with dilute bromine-water, dried over sulphuric acid without the use of a vacuum, and weighed (100 parts tribromphenol correspond to 28.4 parts phenol). It is assumed that the paracresol is first converted by the bromine-water into tribromcresol bromine, and that this is then gradually changed into tribromphenol with the discharge of carbon dioxide. As shown by RUMPF[1] this is not the case, but dibromcresol is chiefly formed instead. This method is therefore not available for this and other reasons. Among the other methods which have been suggested, the following seems to be the most available.

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[1] See Baumann, Pflüger's Arch., Bdd. 12 and 13, and Baumann and Preusse, Zeitschr. f. physiol. Chem., Bd. 3, S. 156.

[2] Schmiedeberg, Arch. f. exp. Path. u. Pharm., Bd. 14.

[3] See G. Hoppe-Seyler, Zeitschr. f. physiol. Chem., Bd. 12. This contains also all references to the literature on this subject.

KOSSLER and PENNY'S[2] method. This method is a modification of MESSINGER and VORTMANN'S[3] volumetric process for estimating phenols. The principle of this process is as follows: The liquid containing phenol is treated with $\frac{n}{10}$ caustic soda until strongly alkaline, warmed on the water-bath in a flask with a glass stopper, and then treated with an excess of $\frac{n}{10}$ iodine solution, the quantity being exactly measured. Sodium iodide is first formed and then sodium hypoiodite, which latter forms tri-iodophenol with the phenol according to the following equation:

$$C_6H_5OH + 3NaIO = C_6H_2I_3.OH + 3NaOH.$$

On cooling acidify with sulphuric acid, and determine by titration with $\frac{n}{10}$ sodium thiosulphate solution the excess of iodine not used. This process is also available for the estimation of paracresol. Each c. c. of the iodine solution used is equivalent to 1.5670 grms. phenol or 1.8018 grms. cresol. As the determination does not give any idea as to the variable proportions of the two phenols, the quantity of iodine used must be calculated as one or the other of the two phenols. In regard to greater details, and especially to precautions, we must refer the reader to the original article of KOSSLER and PENNY.

The methods for the separate determination of the conjugated sulphuric acid and the sulphate-sulphuric acid will be spoken of later in connection with the determination of the sulphuric acid of the urine.

Pyrocatechin-sulphuric Acid (and PYROCATECHIN). This acid was first found in horse's urine in rather large quantities by BAUMANN.[4] It occurs in human urine only in the very smallest quantities, and perhaps not constantly, but it occurs abundantly in the urine after taking phenol, pyrocatechin, or protocatechuic acid.

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[1] Zeitschr. f. physiol. Chem., Bd. 16.

[2] *Ibid.*, Bd. 17.

[3] Ber. d. deutsch. chem. Gesellsch., Bd. 22.

[4] Baumann and Herter, Zeitschr. f. physiol. Chem., Bd. 1.

On an exclusive meat diet this acid does not occur in the urine, and it therefore originates from the vegetable food. It probably originates from the protocatechuic acid, which, according to PREUSSE,[1] passes in part into the urine as pyrocatechin-sulphuric acid. This acid may also perhaps depend on oxidation of phenol within the organism (BAUMANN and PREUSSE[2]).

Pyrocatechin, or O-DIOXYBENZOL, $C_6H_4(OH)_2$, was first observed in the urine of a child (EBSTEIN and J. MÜLLER[3]). The reducing body ALCAPTON, first found by BÖDEKER[4] in human urine and which was considered for a long time as identical with pyrocatechin, was probably *homogentisic acid* or *uroleuic acid* (see below).

Pyrocatechin crystallizes in prisms which are soluble in alcohol, ether, and water. It melts at 102-104° C. and sublimesi n shining plates. The watery solution becomes green, brown, and ultimately black in the presence of alkali and the oxygen of the air. If very dilute ferric chloride is treated with tartaric acid and then made alkaline with ammonia, and this added to a watery solution of pyrocatechin, we obtain a violet or cherry-red liquid which becomes green on saturating with acetic acid. Pyrocatechin is precipitated by lead acetate. It reduces an ammoniacal silver solution at the ordinary temperature and reduces alkaline copper-oxide solutions with heat, but does not reduce bismuth oxide.

A urine containing pyrocatechin, if exposed to the air, especially when alkaline, quickly becomes dark and reduces alkaline copper solutions when heated. In detecting pyrocatechin in the urine it is concentrated when necessary, filtered, boiled with the addition of sulphuric acid to remove the phenols, and repeatedly shaken after cooling with ether. The ether is distilled from the several ethereal extracts, the residue neutralized with barium carbonate and shaken again with ether. The pyrocatechin which remains after evaporating the ether may be purified by recrystallization from benzol.

Hydrochinon, or P-DIOXYBENZOL, $C_6H_4(OH)_2$, often occurs in the urine after the use of phenol (BAUMANN and PREUSSE). The dark color which certain urines, so-called "carbolic urines," take in the air is due to decomposition products. Hydrochinon does not occur as a normal constituent of urine, but after the administration of hydrochinon; according to LEWIN[5] it passes into the urine of rabbits as ethereal-sulphuric acid, as a decomposition product of arbutin.

Hydrochinon forms rhombical crystals which are readily soluble in water, alcohol, and ether. It melts at 169° C. Like pyrocatechin, it easily reduces metallic oxides. It acts like pyrocatechin with alkalies, but is not precipitated with lead acetate. It is oxidized into chinon by ferric chloride and other oxidizing agents, and chinon is detected by its peculiar odor. Hydrochinon-sulphuric acid is detected in the urine by the same methods as pyrocatechin-sulphuric acid.

Indoxyl-sulphuric acid, $C_8H_7NSO_4$ or $C_8H_6N.O.SO_2.OH$, also called URINE INDICAN, formerly called UROXANTHIN (HELLER), occurs as alkali-salt in the urine. This acid is the mother-substance of a great part of the indigo of the urine. The quantity of indigo which can be separated from the urine is considered as a measure of the quantity of indoxyl-sulphuric acid (and indoxyl-glycuronic acid) contained in the urine. This amount, according to JAFFÉ,[1] for man is 5–20 milligrammes per 24 hours. Horse's urine contains about 25 times as much indigo-forming substance as human urine.

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[1] Zeitschr. f. physiol. Chem., Bd. 2.

[2] *Ibid.*, Bd. 3.

[3] Virchow's Arch., Bd. 62.

[4] Zeitschr. f. rat. Med. (3), Bd. 7.

[5] Virchow's Arch., Bd. 92.

Indoxyl-sulphuric acid is derived, as above mentioned (page 489), from indol, which is first oxidized in the body into indoxyl and then is coupled with sulphuric acid. After subcutaneous injection of indol the elimination of indican is considerably increased (JAFFÉ,[2] BAUMANN and BRIEGER[3]). It is also increased by the introduction of orthonitrophenylpropiolic acid in the organism of animals (G. HOPPE-SEYLER[4]). Indol is formed by the putrefaction of proteids, and it is therefore easy to understand why the quantity of indoxyl-sulphuric acid is greater with a meat than with a vegetable diet. The putrefaction of secretions rich in proteid in the intestine explains also the occurrence of indican in the urine during starvation. Gelatine, on the contrary, does not increase the elimination of indican. An abnormally increased elimination of indican occurs in such diseases as obstruct the small intestine, causing an increased putrefaction, thus producing an abundant formation of indol. Such an increased elimination of indican occurs on tying the small intestine of a dog, but not the large intestine (JAFFÉ[5]).

The elimination of indican may also be caused by the putrefaction of proteids in other organs and tissues of the body besides the intestine. An increased elimination of indican has been observed in many diseases[6], and in these cases the quantity of phenol eliminated is generally increased. A urine rich in phenol is not always rich in indican.

The potassium-salt of indoxyl-sulphuric acid, which was prepared by BAUMANN and BRIEGER[7] from the urine of a dog fed on indol, crystallizes in colorless, shining plates or leaves which are easily soluble in water but less readily in alcohol. It is split by mineral acids into sulphuric acid and indoxyl. The latter without access of air passes into a red compound, indoxyl-red, but in the presence of oxidizing reagents is converted into indigo-blue: $2C_8H_7NO + 2O = C_{16}H_{10}N_2O_2 + 2H_2O$. The detection of indican is based on this last fact.

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[1] Pflüger's Arch., Bd. 3.

[2] Centralbl. f. d. med. Wissensch., 1872.

[3] Zeitschr. f. physiol. Chem., Bd. 3.

[4] *Ibid.*, Bdd. 7 and 8.

[5] Virchow's Arch., Bd. 70.

[6] See Jaffé, Pflüger's Arch., Bd. 3; Senator, Centralbl. f. d. med. Wissensch., 1877; G. Hoppe-Seyler, Zeitschr. f. physiol. Chem., Bd. 12 (contains older literature); also Berl. klin. Wochenschr., 1892.

[7] Zeitschr. f. physiol. Chem., Bd. 3, S. 254.

For the rather complicated preparation of indoxyl-sulphuric acid as potassium-salt from urine the reader is referred to other text-books. For the detection of indican in urine in ordinary cases the following method of JAFFÉ,[1] which also serves as an approximate test for the quantity of indican, is sufficient.

JAFFÉ'S *Indican Test.* 20 c. c. of urine are treated in a test-tube with 2–3 c. c. chloroform and mixed with an equal volume of concentrated hydrochloric acid. Immediately after a concentrated chloride-of-lime solution or a $\frac{1}{2}\%$ potassium permanganate solution is added drop by drop, and after each drop the mixture is thoroughly shaken. The chloroform is gradually colored faintly or strongly blue. An excess of oxidizing reagent, especially chloride of lime, interferes with the reaction and must therefore be avoided. The test is repeated with somewhat varying amounts of oxidizing material until a point is found at which the maximum coloration of the chloroform takes place. From the intensity of the color the quantity of indigo is determined.

OBERMAYER[2] uses fuming hydrochloric acid containing 2–4 parts ferric chloride per litre to decompose the indican and to oxidize the indoxyl. The urine is first precipitated with not too much lead acetate and the filtrate shaken for 1–2 minutes with an equal volume of the above hydrochloric acid. The indigo blue is taken up by chloroform in this case also.

According to ROSIN[3] some indigo-red is always formed besides the indigo-blue in JAFFÉ'S indican test. Greater quantities of indigo-red are formed when the decomposition of the indican takes place in the warmth (see ROSENBACH'S urine test).

An exact determination of the amount of indigo in urine is very rarely made. The methods suggested for this purpose are very complicated, and even then they are not quite accurate; therefore the reader is referred to other text-books for their description.

Indol seems also to pass into the urine as a glycuronic acid, *indoxyl-glycuronic acid* (SCHMIEDEBERG[4]). Such an acid has been found in the urine of animals after the administration of the sodium-salt of o-nitrophenylpropiolic acid (G. HOPPE-SEYLER[5]).

Skatoxyl-sulphuric Acid, $C_9H_9NSO_4$ or $C_9H_8.N.O.SO_2.OH$.

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[1] Pflüger's Arch., Bd. 3.

[2] Wien. klin. Wochenschr., 1890.

[3] Virchow's Arch., Bd. 123.

[4] Arch. f. exp. Path. u. Pharm., Bd. 14.

[5] Zeitschr. f. physiol. Chem., Bdd. 7 and 8.

The potassium-salt of this acid seems to occur generally in human urine as a chromogen, which yields a red or violet coloring matter on decomposing with strong acids, and an oxidizing reagent. This salt has been prepared by OTTO[1] from diabetic human urine. Little is known of the quantity of this skatol-chromogen, to which probably also the skatoxyl-glycuronic acid must be counted, under physiological and pathological conditions.

Skatoxyl-sulphuric acid originates from skatol formed by putrefaction in the intestine, which is coupled with sulphuric acid after oxidation into skatoxyl. That skatol introduced into the body passes partly as an ethereal-sulphuric acid into the urine has been shown by BRIEGER.[2] Indol and skatol act differently, at least in dogs; indol producing a considerable amount of ethereal-sulphuric acid, while skatol only gives a small quantity (MESTER[3]). Skatol seems partly to pass into the urine as a *skatoxyl-glycuronic acid.*

The potassium-salt of skatoxyl-sulphuric acid is crystalline; it dissolves in water, but with difficulty in alcohol. A watery solution becomes deep violet with ferric chloride, and red with concentrated nitric acid. The salt is decomposed by concentrated hydrochloric acid with the separation of a red precipitate. The nature of this red coloring matter produced by the decomposition of skatoxyl-sulphuric acid is not well known; neither is the relationship existing between this and other red coloring matters in the urine known. On distillation with zinc-dust the skatol chromogen yields skatol.

Urines containing skatoxyl are colored dark red to violet by JAFFÉ's indican test even on the addition of hydrochloric acid; with nitric acid they are colored cherry-red, and red on warming with ferric chloride and hydrochloric acid. The coloring matter which yields skatol with zinc-dust may be removed from the urine by ether. Urines rich in skatoxyl darken when allowed to stand in the air from the surface downward, and may become reddish, violet, or nearly black. ROSIN[4] is of the opinion that no skatol-chromogen exists in human urine, and that the observations made heretofore were due to a confusion with indigo-red or urorosein.

SALKOWSKI[5] has shown that the occurrence of *skatol-carbonic acid,* C[illegible]H[illegible].N.COOH, in normal urine is probable. This is also a putrefaction product.

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[1] Pflüger's Arch., Bd. 33.

[2] Ber. d. deutsch. chem. Gesellsch., Bd. 12, and Zeitschr. f. physiol. Chem., Bd. 4, S. 414.

[3] Zeitschr. f. physiol. Chem., Bd. 12.

[4] L. c.

[5] Zeitschr. f. physiol. Chem., Bd. 9.

Aromatic Oxyacids. In the putrefaction of proteids in the intestine, *paraoxyphenyl-acetic acid*, $C_6H_4(OH).CH_2COOH$, and *paraoxyphenyl-propionic acid*, $C_6H_4(OH).C_2H_4.COOH$, are formed from tyrosin as intermediate steps, and these in great part pass unchanged into the urine. They were first detected by BAUMANN.[1] The quantity of these acids is usually very small. They are increased by the same circumstances as phenol, especially in acute phosphorus-poisoning, in which the increase is considerable. A small portion of these oxyacids is combined with sulphuric acid.

Besides these two oxyacids which regularly occur in human urine we sometimes have other oxyacids in urines. To these belong *homogentisic acid* and *uroleucic acid*, which form the specific constituents of the urine in most cases of alcaptonuria, *oxymandelic acid*, found by SCHULTZEN and RIESS[2] in urine in acute atrophy of the liver, *oxyhydroparacumaric acid*, found by BLENDERMANN[3] in the urine on feeding rabbits with tyrosin, *gallic acid*, which, according to BAUMANN,[4] sometimes appears in horse's urine, and *kynurenic acid* (oxychinolincarbonic acid), which has only been found up to the present time in dog's urine. The first two above-mentioned oxyacids, and also homogentisic and uroleucic acids, will be treated of here.

Paraoxyphenylacetic acid and **p-oxyphenylpropionic acid** are crystalline and are both soluble in water and in ether. The first melts at 148° C. and the other at 125° C. Both give a beautiful red coloration on being warmed with MILLON'S reagent.

To detect the presence of these oxyacids proceed in the following way (BAUMANN): Warm the urine for a while on the water-bath with hydrochloric acid, in order to drive off the volatile phenols. After cooling shake three times with ether, and then shake the ethereal extracts with dilute soda solution, which dissolves the oxyacids, while the residue of the phenols soluble in ether remains. The alkaline solution of the oxyacids is now faintly acidified with sulphuric acid, shaken again with ether, the ether removed and allowed to evaporate, the residue dissolved in a little water, and the solution tested with MILLON'S reagent. The two oxyacids are best differentiated by their different melting-points. The reader is referred to other works for the method of isolating and separating these two oxyacids.

Homogentisic acid, $C_8H_8O_4$ or $C_6H_3(OH)_2.CH_2.COOH$. This acid was detected by WOLKOW and BAUMANN.[5] They isolated it from the urine in a case of alcaptonuria (see below) and showed that the characteristics of so-called alcaptonuric urine in this case were due to this acid. This acid has later been found in other cases of alcaptonuria by EMBDEN,[1] GARNIER and VOIRIN,[2] and OGDEN.[3] *Glycosuric acid*, isolated from alcaptonuric urine by MARSHALL[4] and recently by GEYGER,[5] seems to be identical with homogentisic acid. Tyrosin is considered as the mother-substance of this acid. On the introduction of tyrosin in persons with alcaptonuria, WOLKOW and BAUMANN and EMBDEN observed a greater or less increase in the quantity of homogentisic acid in the urine. According to WOLKOW and BAUMANN this acid is formed from the tyrosin by abnormal putrefactive processes in the upper part of the intestine.

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[1] Ber. d. deutsch. chem. Gesellsch., Bdd. 12 and 13, and Zeitschr. f. physiol. Chem., Bd. 4.

[2] Chem. Centralbl., 1869.

[3] Zeitschr. f. Physiol. Chem., Bd. 6, S. 257.

[4] *Ibid.*, Bd. 6, S. 193.

[5] *Ibid.*, Bd. 15.

Homogentisic acid is that dioxyphenyl-acetic acid derived from hydrochinon. On fusion with potash it yields gentisic acid (hydrochinon-carbonic acid) and hydrochinon. When introduced into the intestinal tract of dogs it is in part converted into tolu-hydrochinon, which is eliminated in the form of ethereal-sulphuric acid. Homogentisic acid has recently been prepared synthetically by BAUMANN and FRANKEL,[6] starting with gentisic aldehyde.

Homogentisic acid crystallizes with 1 mol. water in large, transparent prismatic crystals, which become non-transparent at the temperature of the room with the loss of water of crystallization. They melt at 146.5–147° C. They are soluble in water, alcohol, and ether, but nearly insoluble in chloroform and benzol. Homogentisic acid is optically inactive and non-fermentable. Its watery solution has the properties of so-called alcaptonuric urine. It becomes greenish brown from the surface downward on the addition of very little caustic soda or ammonia with excess of oxygen, and on stirring it becomes quickly dark brown or black. It reduces alkaline copper solutions with even slight heat, and ammoniacal silver solutions immediately in the cold. It does not reduce alkaline bismuth solutions. Among the salts of this acid we must mention the lead salt containing water of crystallization and 34.79% Pb. This salt melts at 214–215° C.

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[1] Zeitschr. f. physiol. Chem., Bdd. 17 and 18.

[2] Arch. de Physiol., (5) Tome 4.

[3] Zeitschr. f. physiol. Chem., Bd. 20.

[4] See Maly's Jahresber., Bd. 17.

[5] Pharm. Ztg., 6 Aug. 1892, S. 488. Cited from Embden, Zeitschr. f. physiol. Chem., Bd. 18.

[6] Zeitschr. f. Physiol. Chem., Bd. 20.

In preparing this acid the strongly acidified urine is shaken with ether. The residue obtained on the distillation of the ether is dissolved in water, the solution heated to boiling and treated with a lead acetate solution (1 : 5), and the brown resinous precipitate quickly separated by filtration. The lead salt gradually crystallizes from the filtrate. This is decomposed by sulphuretted hydrogen and the acid obtained as crystals from the filtrate after carefully concentrating the filtrate finally in vacuo.

In regard to the quantitative estimation we proceed according to the suggestion of BAUMANN by titrating the acid with a $\frac{n}{10}$ silver solution. As regards details of this method we must refer the reader to the original publication.[1]

Uroleucic acid, $C_9H_{10}O_5$, is, according to HUPPERT,[2] probably at rioxyphenolpropionic acid, $(HO)_3.C_6H_2CH_2.CH_2.COOH$. This acid was first prepared by KIRK[3] from the urine of children with alcaptonuria. According to WOLKOW and BAUMANN it is not identical with homogentisic acid, and has a melting-point of 133° C. Otherwise, in regard to its behavior with alkalies, with access of air, and also with alkaline copper solutions and ammoniacal silver solutions, it is similar to homogentisic acid. In chemical properties it is very similar to gallic acid.

Urinary Pigments and Chromogens. The yellow color of normal urine depends apparently upon several coloring matters which have not been isolated and studied. Besides these bodies, UROBILIN sometimes occurs in fresh normal urine, but by no means always. Instead of urobilin, normal urine often contains a mother-substance of the same, a chromogen or UROBILINOGEN, from which the urobilin is gradually formed by oxidation on allowing the urine to stand exposed to the air (JAFFÉ,[4] DISQUÉ,[5] and others). Besides this chromogen, urine contains various other bodies from which coloring matters may be produced by the action of chemical agents. Humin substances (perhaps in part from the carbohydrates of the urine) may be formed by the action of acids (v. UDRÁNSZKY[6]) without regard to the fact that such substances may sometimes originate from the reagents used, as from impure amyl-alcohol (v. UDRÁNSZKY and HOPPE-SEYLER[7]). To these humin bodies developed by the action of acid in normal urine when exposed to the air must be added the urophain of Heller,[1] the various uromelanins, and other bodies described by different investigators (Plos'z,[2] Thudichum,[3] Schunck[4]). Indigo-blue (uroglaucin of Heller, urocyanin, cyanurin, and other coloring matters of older investigators[5]) is split off from the indoxyl-sulphuric acid or indoxyl-glycuronic acid. Red coloring matters may be formed from the conjugated indoxyl and skatoxyl acids, and urohodin (Heller), urorubin (Plos'z), urohæmatin (Harley[6]), and perhaps also urorosein (Nencki and Sieber[7]) probably have such an origin.

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[1] Zeitschr. f. physiol. Chem., Bd. 16.

[2] Hüppert-Neubauer, Analyse des Harns, 10. Aufl., S. 246.

[3] Brit. Med. Journal, 1886 and 1888; Journal of Anat. and Physiol., Vol. 23.

[4] Centralbl. f. d. med. Wissensch, 1868 and 1869; Virchow's Arch., Bd. 47.

[5] Zeitschr. f. physiol. Chem., Bd. 2.

[6] *Ibid.*, Bdd. 11 and 12.

[7] Hoppe-Seyler, Ber. d. deutsch. Chem. Gellsch., Bd. 18, and v. Udránszky, Zeitschr. f. physiol Chem., Bd. 13.

We cannot enter into too many details of the different coloring matters obtained as decomposition products from normal urine; and as the preformed physiological coloring matters of urine have not been closely studied, we can only discuss the most carefully investigated urinary pigment, urobilin.

Urobilin was first prepared from urine by Jaffé.[8] This coloring matter occurs in urine especially in fevers, and it is therefore designated febrile urobilin by MacMunn.[9] The urobilin occurring in normal urine is somewhat different from an optical standpoint from the above, and is called normal urobilin by MacMunn. As above stated, a mother-substance of urobilin, a urobilinogen, occurs in the urine, from which urobilin is produced by the action of the air.

Many investigators claim that urobilin is identical with hydrobilirubin (Maly) and corresponds to the composition $C_{32}H_{40}N_4O_7$. Also, that urobilin is formed by a reduction of bilirubin in the intestine. The correctness of this view is disputed by others (MacMunn, Le Nobel[10]). According to MacMunn, hydrobilirubin and urinary urobilin are not identical bodies, because he obtained normal urobilin by the action of peroxide of hydrogen upon a solution of hæmatin in alcohol containing sulphuric acid.

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[1] Heller's Arch. (2), Bd. 1. Cited from Huppert-Neubauer, S. 326.

[2] Zeitschr. f. physiol. Chem., Bd. 8.

[3] Brit. Med. Journal, Vol. 201 (1864), and Journ. f. prakt. Chem., Bd. 104.

[4] Cited from Huppert-Neubauer, S. 509.

[5] *Ibid.*, S. 161.

[6] In regard to this and other red pigments see Huppert-Neubauer, S. 557-598.

[7] Journ. f. prakt. Chem. (2), Bd. 26.

[8] L. c.

[9] Proc. Roy. Soc., Vols. 31 and 35; Ber. d. deutsch. chem. Gesellsch., Bd. 14; Journal of Physiol., Vols. 6 and 10. In regard to different urobilins, see Bogomoloff, Maly's Jahresber., Bd. 22, and Eichholz, Journal of Physiol., Vol. 14.

[10] See Chapter VIII, on Bile-pigments.

Pigments similar to urobilin, though not identical, have been obtained from the biliary and blood coloring matters. Besides hydrobilirubin, prepared by MALY from bilirubin, STOKVIS[1] obtained a choletelin from a biliary pigment, cholecyanin, by the action of zinc chloride and tincture of iodine, or by boiling with a little lead peroxide. This choletelin acts like urobilin, but that obtained from bilirubin by the action of nitric acid does not. Bodies similar to urobilin have also been obtained by HOPPE-SEYLER[2] by the reduction of hæmatin and hæmoglobin with zinc and hydrochloric acid; by LE NOBEL[3] by treating an acid-alcoholic or alkaline solution of hæmatoporphyrin with tin or zinc; and lastly by NENCKI and SIEBER[4] by treating hæmatoporphyrin with zinc and hydrochloric acid. From the observations of LE NOBEL and NENCKI and SIEBER it follows that these pigments artificially prepared from the blood-coloring matters are not identical with urinary urobilin, even though they are closely related from an optical standpoint. It must be left undecided whether these bodies are identical with each other or with the urinary urobilin, or if the observed difference is only due to a contamination with other bodies.

We have numerous observations on the elimination of urobilin in disease, especially by JAFFÉ, DISQUÉ, DREYFUSS-BRISSAC, GERHARDT, G. HOPPE-SEYLER,[5] and others. Because of our imperfect knowledge of the urobilin of the urine and the UROBILINOIDIN (this name has been given by LE NOBEL to the substance similar to urobilin artificially prepared by him) it is difficult to say anything positive in regard to the occurrence of urobilin in the urine in disease. During the absorption of large blood extravasations, as also in diseases connected with destruction of the blood-corpuscles or of the appearance of methæmoglobin in the blood-plasma, the urine becomes dark in color, which generally depends upon an increased elimination of urobilin. The question whether it depends on an increased elimination of urinary urobilin or, as is more probable, upon the urobilinoidin produced from the blood-coloring matters is still doubtful. In icterus the elimination of urobilin is often increased, and indeed cases occur in which urobilin is almost the only coloring matter which can be detected in the urine (UROBILINICTERUS). In these cases we are probably dealing with a urobilinoid substance produced from bilirubin in the intestinal tract by reduction.

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[1] See Chapter VIII.

[2] Ber. d. deutsch. chem. Gesellsch., Bd. 7.

[3] Pflüger's Arch., Bd. 40.

[4] Monatshefte f. Chem., Bd. 9, and Arch. f. exp. Path. u. Pharm., Bd. 24.

[5] In regard to the literature on this subject we refer the reader to D. Gerhardt, "Ueber Hydrobilirubin und seine Beziehungen zum Ikterus" (Berlin 1889), and also G. Hoppe-Seyler, Virchow's Arch., Bd. 124.

The urobilin obtained from fever urine is, according to JAFFÉ, amorphous, red, dingy red, or reddish yellow, according to the method of preparation. It dissolves easily in alcohol, amyl-alcohol, and chloroform, but less readily in ether. It is less soluble in water, but the solubility is augmented in the presence of a neutral salt. It may be precipitated from a solution saturated with ammonium sulphate by the addition of sulphuric acid (MÉHY[1]). It is soluble in alkalies, and is incompletely precipitated from the alkaline solution by the addition of acid. It is partly dissolved by chloroform from an acid (watery-alcoholic) solution; alkali solutions remove the urobilin from the chloroform. Alkaline solutions of urobilin give insoluble combinations with salts of the heavy metals, such as zinc and lead. Urobilin does not give GMELIN's test for bile-pigments.

Neutral alcoholic urobilin solutions are in strong concentration brownish yellow, in great dilution yellow or rose-colored. They have a strong green fluorescence. The acid-alcoholic solutions are brown, reddish yellow, or rose-red, according to concentration. They are not fluorescent, but show a faint absorption-band, γ, between b and F, which borders on F, or in greater concentration extends over F. The alkaline solutions are brownish yellow, yellow, or (the ammoniacal) yellowish green, according to concentration. If some zinc-chloride solution is added to an ammoniacal solution, it becomes red and shows a beautiful green fluorescence. This solution, as also that made alkaline with fixed alkalies, shows a darker and more sharply defined band, δ, almost midway between b and F.

The urobilins obtained from the urine by MACMUNN by another method, and that obtained by JAFFÉ, differ from each other mainly in the following: A solution of normal urobilin becomes deeper red with soda, while febrile urobilin becomes yellow. The band γ of normal urobilin disappears on the addition of alkali, while the corresponding band of febrile urobilin moves towards the left. The ethereal solution of febrile urobilin shows two faint absorption-bands on each side of *D* which are not to be seen in the watery solution nor in the urine. Febrile urobilin is a brownish-red and the normal a yellowish-brown powder. Febrile urobilin is, according to MACMUNN, converted into normal urobilin by potassium permanganate.

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[1] Journal de pharm. et de chim., 1878. Cited from Maly's Jahresber., Bd. 8, S. 269.

In preparing urobilin from normal urine, precipitate the urine with basic lead acetate (JAFFÉ), wash the precipitate with water, dry at the ordinary temperature, then boil it with alcohol, and decompose it when cold with alcohol containing sulphuric acid. The filtered alcoholic solution is diluted with water, saturated with ammonia, and then treated with zinc-chloride solution. This new precipitate is washed free from chlorine with water, boiled with alcohol, dried, dissolved in ammonia, and this solution precipitated with sugar of lead. This precipitate, which is washed with water and boiled with alcohol, is decomposed by alcohol containing sulphuric acid, the filtered alcoholic solution is mixed with ½ vol. chloroform, diluted with water, and shaken repeatedly, but not too energetically. The urobilin is taken up by the chloroform. This last is washed once or twice with a little water and then distilled, leaving the urobilin, which is purified from a contaminating red coloring matter by means of ether.

According to JAFFÉ, the coloring matter can be directly precipitated from a fever urine rich in urobilin by ammonia and zinc chloride, and this precipitate treated as above. MÉHY faintly acidifies the urine with sulphuric acid (1–2 grms. per litre), then saturates with ammonium sulphate, washes the precipitate on a filter with an acidified ammonium-sulphate solution, presses the filter, and extracts the coloring matter with absolute alcohol at a gentle heat after the addition of a few drops of ammonia. MACMUNN precipitates the urine with sugar of lead and basic lead acetate, decomposes the precipitate with acidified alcohol, dilutes the solution with water, shakes with chloroform, evaporates this last, and dissolves the residue repeatedly with chloroform. The method of preparation, according to MACMUNN, is the same for both urobilins, the normal and the febrile.

The color of the acid or alkaline solution, the beautiful fluorescence of the ammoniacal solution treated with zinc chloride, and the absorption-bands of the spectrum, all serve as means of detecting urobilin. In fever urines the urobilin may be detected directly or after the addition of ammonia and zinc chloride by its spectrum. It may also be detected sometimes in normal urine directly or after the urine has stood exposed to the air until the chromogen has been converted into urobilin. If it cannot be detected by means of the spectroscope, then the urine may be treated with a mineral acid and shaken with ether. The ethereal solution, directly or after concentration, may be tested with the spectroscope. It is often better to dissolve the residue, after the evaporation of the ether, in absolute alcohol, and use this for the spectroscopic investigation. According to SALKOWSKI, the urobilin may be directly extracted by gently shaking with ether free from alcohol. If the urobilin cannot be detected by the above-described methods, then precipitate the urine with basic lead acetate, decompose the precipitate with acidified alcohol, test this solution or extract the coloring matter by diluting with water and shaking with chloroform.

In the quantitative estimation of urobilin we proceed as follows, according to G. HOPPE-SEYLER:[1] 100 c. c. of the urine are acidified with sulphuric acid and saturated with ammonium sulphate. The precipitate is collected on a filter after some time, washed with a saturated solution of ammonium sulphate, and repeatedly extracted with equal parts alcohol and chloroform after pressing. The filtered solution is treated with water in a separatory funnel until the chloroform separates well and becomes clear. The chloroform solution is evaporated on the water-bath in a weighed beaker, the residue dried at 100° C., and then extracted with ether. The ethereal extract is filtered, the residue on the filter dissolved in alcohol, and transferred to the beaker and evaporated, then dried and weighed. According to this method G. HOPPE-SEYLER found 0.08–0.14 grm. urobilin in one day's urine of a healthy person, or an average of 0.123 grm.

The real yellow pigment of urine has been only slightly investigated. This pigment has been called UROCHROM by GARROD,[2] which name has been used by THUDICHUM earlier to designate a mixture of pigments and other substances. The pigment isolated by GARROD by a rather complicated method was amorphous brown, very easily soluble in water and ordinary alcohol, less soluble in absolute alcohol, and insoluble in ether, chloroform, and benzol. It shows no absorption-bands, and does not fluoresce on the addition of ammonia and zinc chloride.

Uroerythrin is that coloring matter which often colors the urinary sediment (*sedimentum lateritium*) beautifully red. It occurs especially in fevers and other diseases, but it is also found in the urine of perfectly healthy persons. Its solution is colored green by alkalies and, according to ZOJA,[3] shows a strong absorption beginning between D and E and extending to F. This absorption consists of two bands, of which the one at F is the stronger. Uroerythrin dissolves readily in amyl alcohol. GARROD[4] has suggested a method for obtaining uroerythrin, and has given further contributions for the detection of the same. Attention is called especially to the well-known property of uroerythrin of being bleached on exposure to light.

[1] Virchow's Arch., Bd. 124.

[2] Proc. of Roy. Soc., Vol. 55, 1894. See also Thudichum, Brit. Med. Journal, 1864, Vol. 2; Journal f. prakt. Chem., Bd. 104.

[3] Arch. ital. di clinica med., 1893; also Centralbl. f. d. med. Wissensch., 1892.

[4] Journal of Physiol., Vol. 17.

Volatile fatty acids, such as formic acid, acetic acid, and perhaps also butyric acid, occur under normal conditions in human urine (v. Jaksch [1]), also in that of dogs and herbivora (Schotten [2]). The acids poorest in carbon, such as formic acid and acetic acid, are more constant in the body than those richer in carbon, and therefore the relatively greater part of these pass unchanged into the urine (Schotten). Normal human urine contains besides these bodies others which yield acetic acid when oxidized by potassium dichromate and sulphuric acid (v. Jaksch). The quantity of volatile fatty acids in normal urine is, according to v. Jaksch, 0.008–0.009 grm. per 24 hours, and according to v. Rokitansky,[3] 0.054 grm. The quantity is increased by exclusive farinaceous food (Rokitansky), also in fever and in certain diseases of the liver (v. Jaksch). It is also increased in leucæmia and in many cases of diabetes (v. Jaksch). Large amounts of volatile fatty acids are produced in alkaline fermentation of the urine, and the quantity is 6–15 times as large as in normal urine (Salkowski [4]).

Paralactic Acid. It is claimed that this acid occurs in the urine of healthy persons after very fatiguing marches (Colasanti and Moscatelli [5]). It is found in larger amounts in the urine in acute phosphorus-poisoning or acute yellow atrophy of the liver (Schultzen and Riess [6]). According to the investigations of Hoppe-Seyler and Araki,[7] lactic acid, besides sugar, passes into the urine as soon as the supply of oxygen is decreased in any way. Minkowski [8] has shown that lactic acid occurs in the urine in large quantities on the extirpation of the liver of birds.

Glycero-phosphoric acid occurs as traces in the urine, and it is probably a decomposition product of lecithin. The occurrence of *succinic acid* in normal urine is the subject of discussion.

Carbohydrates and Reducing Substances in the Urine. The occurrence of *grape-sugar* as traces in normal urine is highly probable, as the investigations of Brücke, Abeles, and v. Udránszky show. The last investigator has also shown the habitual occurrence of carbohydrates in the urine, and their presence has been positively proved by the investigations of Baumann and Wedenski, and especially by Baisch. Besides glucose normal urine contains, according to Baisch, another not well-studied variety of sugar, probably isomaltose, and besides this a dextrin-like carbohydrate (animal gum), as shown by Landwehr, Wedenski, and Baisch.[9]

Besides traces of sugar and the previously mentioned reducing substances, uric acid and creatinin, the urine contains still other reducing substances. These last are probably (Flückiger [10]) conjugated combinations of *glycuronic acid*, $C_6H_{10}O_7$, which closely resembles sugar. The reducing power of normal urine corresponds, according to various investigators, to 1.5–5.96 p. m. grape-sugar.[1]

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[1] Zeitschr. f. physiol. Chem., Bd. 10.

[2] *Ibid.*, Bd. 7.

[3] Wien. med. Jahrb., 1887; cited from Maly's Jahresber., Bd. 17.

[4] Zeitschr. f. physiol. Chem., Bd. 13.

[5] Moleschott's Untersuch. zur Naturlehre, Bd. 14.

[6] Chem. Centralbl., 1869.

[7] Zeitschr. f. physiol. Chem., Bdd. 15, 16, 17, and 19. Irisawa, *ibid.*, Bd. 17.

[8] Arch. f. exp. Path. u. Pharm., Bdd. 21 and 31.

[9] Zeitschr. f. physiol. Chem., Bdd. 18, 19, and 20; Treupel, *ibid.*, Bd. 16. These articles contain references to the work of other investigators.

[10] Zeitschr. f. physiol. Chem., Bd. 9.

Glycuronic Acid, $C_6H_{10}O_7$ or $CHO.(CH.OH)_4.COOH$. This acid may be converted into saccharic acid, $C_6H_{10}O_8$, by the action of bromine (THIERFELDER[2]), and it seems to occupy an intermediate position between this acid and gluconic acid, $C_6H_{12}O_7$. It is a derivative of glucose, and FISCHER and PILOTY[3] have prepared it synthetically by the reduction of saccharo-lactonic acid. Further reduction yields gulonic acid lacton (THIERFELDER). Glycuronic acid is an intermediate metabolic product, and it only occurs in the urine when it is protected from combustion in the animal body by combining with other bodies. Such conjugated combinations with indoxyl, skatoxyl, and phenols occur probably normally in very small quantities in human urine. This acid as conjugated glycuronic acids passes in large quantities into the urine after the administration of various therapeutic agents or certain other substances. Thus SCHMIEDEBERG and MEYER[4] found campho-glycuronic acid in the urine after partaking of camphor, and v. MERING[5] showed the presence of urochloralic acid (see Accidental Constituents of the Urine) after the administration of chloral hydrate. According to SCHMIEDEBERG,[6] glycuronic acid seems to occur in cartilage because it is contained in chondrosin, a cleavage product of chondroitic-sulphuric acid. It is also found in the artist's color "jaune indien," which contains the magnesium-salt of euxanthonic acid (euxanthon-glycuronic acid). On heating this acid with water to 120–125° C. it splits into euxanthin and glycuronic acid, and it is the most available material for the preparation of glycuronic acid (THIERFELDER). Another acid, isomeric with the ordinary glycuronic acid, has been found in the urine in certain cases (see Accidental Constituents of the Urine).

Glycuronic acid is not crystalline, but is obtained only as a syrup. It dissolves in alcohol and is easily soluble in water. If the watery solution is boiled for an hour, the acid is in part (20%) converted into the anhydride GLYCURON, $C_6H_8O_6$, which is crystalline, soluble in water, but insoluble in alcohol. The alkali salts of this acid are crystalline. The neutral barium salt is amorphous, soluble in water, but is precipitated by alcohol. If a concentrated solution of the acid is saturated with barium hydrate, the basic barium salt separates. The neutral lead salt is soluble in water, but the basic salt is, on the contrary, insoluble. The acid is dextrogyrate and reduces copper, silver, and bismuth salts. It does not ferment with yeast. Glycuronic acid gives the furfurol reaction and acts like a pentose when tested with the phloroglucin-hydrochloric-acid test. With phenylhydrazin potassium glycuronate gives a flaky yellow precipitate of microscopic needles which melt at 114–115° C. (THIERFELDER). The statements in regard to the behavior of glycuronic acid with this test are very contradictory.[1]

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[1] See Huppert-Neubauer, S. 72.

[2] The works of Thierfelder on glycuronic acid are found in Zeitschr. f. physiol. Chem., Bdd. 11, 13, and 15.

[3] Ber. d. deutsch. chem. Gesellsch., Bd. 24, S. 521.

[4] Zeitschr. f. physiol. Chem., Bd. 3.

[5] *Ibid.*, Bd. 6.

[6] Arch. f. exp. Path. u. Pharm., Bd. 28.

All conjugated glycuronic acids are lævorotatory, while glycuronic acid itself is dextrorotatory. They are split into glycuronic acid and the several other groups by the addition of water. A few of the conjugated glycuronic acids, such as the urochloralic acid, reduce copper oxide and certain other metallic oxides in alkaline solution, and therefore they may interfere with the detection of sugar in the urine.

Glycuronic acid may be prepared from urochloralic acid or campho-glycuronic acid by boiling with a mineral acid. It may be prepared more easily by heating euxanthonic acid with water in PAPIN's digester to 120–125° C. for an hour and evaporating the watery solution at + 40° C. The anhydride which crystallizes gradually is removed, the mother-liquor diluted with water and boiled for a time to convert a second portion of acid into anhydride, and then evaporated at about + 40° C. This is continued until nearly all the acid is converted into anhydride. The anhydride may then be further purified.

Organic combinations containing sulphur of unknown kind, which may in small part consist of *sulphocyanides*, 0.04 (GSCHEIDLEN[2])–0.11 p. m. (I. MUNK),[3] *cystin*, or bodies related to it, and *protein bodies*, are found in human as well as in animal urines. LANG[4] has shown that nitriles of the fatty series when united with hydrocyanic acid in the animal body are converted into sulphocyanides, and pass as such into the urine. This sulpchocyanide originates, it seems, from the readily cleavable, non-oxidizable sulphur of the proteid bodies, which, as PASCHELES (*ibid.*) has shown, readily converts potassium cyanide into alkali sulphocyanide in an alkaline reaction and at the temperature of the body. The amido-acids of the fatty series in the body are probably oxidized to nitriles, which are then transformed into sulphocyanides by the sulphur of the proteids. The sulphur of these mostly unknown combinations has been called "neutral," to differentiate it from the "acid" sulphur of the sulphate and ethereal-sulphuric acids (Salkowski[1]). The neutral sulphur in normal urine as determined by Salkowski is 15%, by Stadthagen[2] 13.3-14.5%, and by Lépine[3] 20% of the total sulphur. In starvation, according to Fr. Müller,[4] the absolute and relative quantities increase. According to Heffter[5] the quantity is greater with a bread diet than with a meat diet. Excessive muscular exercise increases the elimination of the acid as well as the neutral sulphur; still, according to Beck and Benedikt,[6] the increase in neutral sulphur takes place earlier. According to Presch[7] sulphur when introduced in the body increases the elimination of neutral sulphur; indeed, about one fourth of the sulphur absorbed in the elementary state passes into organic combinations, not oxidizable by nitric acid alone. According to the investigations of W. Smith[8] it is probable that the most unoxidizable part of the neutral sulphur occurs as sulpho-acids. An increased elimination of neutral sulphur has been observed in various diseases, such as pneumonia, icterus, and cystinuria.

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[1] In regard to literature see Hammarsten, Zeitschr. f. physiol. Chem., Bd. 19, S. 30, and Roos, *ibid.*, Bd. 15, S. 525.

[2] Pflüger's Arch., Bd. 14.

[3] Virchow's Arch., Bd. 69.

[4] Arch. f. exp. Path. u. Pharm., Bd. 34.

The total quantity of sulphur in the urine is determined by fusing the solid urinary residue with saltpetre and caustic alkali. The quantity of neutral sulphur is determined as the difference between the total sulphur and the sulphur of the sulphate and ethereal-sulphuric acids.

Sulphuretted hydrogen occurs in urine only under abnormal conditions or as a decomposition product. Sulphuretted hydrogen may be produced from the neutral sulphur of the organic substances of the urine by the action of certain bacteria (Fr. Müller,[9] Salkowski[10]). Other investigators have given *hyposulphites* as the source of the sulphuretted hydrogen. The occurrence of hyposulphites in normal human urine, which is asserted by Heffter,[11] is disputed by Salkowski[12] and Presch.[13] Hyposulphites occur constantly in cat's urine and, as a rule, also in dog's urine.

Organic combinations containing phosphorus (glycero-phosphoric acid, etc.), which yield phosphoric acid on fusing with saltpetre and caustic alkali, are also found in urine (Lépine, Eymonnet, and Aubert[14]).

Enzymes of various kinds have been isolated from the urine. Among these we may mention *pepsin* (Brücke[15] and others), *diastatic enzyme* (Cohnheim[16] and others). The occurrence of rennin and trypsin in the urine is doubtful.[17]

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[1] Virchow's Arch., Bd. 58, and Zeitschr. f. physiol. Chem., Bd. 9.

[2] Virchow's Arch., Bd. 100.

[3] Compt. rend., Tomes 91 and 97.

[4] Berlin. klin. Wochenschr., 1887.

[5] Pflüger's Arch., Bd. 38.

[6] Maly's Jahresber., Bd. 22, S. 223.

[7] Virchow's Arch., Bd. 119.

[8] Zeitschr. f. physiol. Chem., Bd. 17.

[9] Berlin. klin. Wochenschr., 1887.

[10] *Ibid.*, 1888.

[11] Pflüger's Arch., Bd. 38.

[12] *Ibid.*, Bd. 39.

[13] Virchow's Arch., Bd. 119.

[14] Compt. rend., Tome 98, and Compt. rend. de la soc. de Biol., 1882 and 1884.

[15] Wien. Sitzungsber., Bd. 43.

[16] Virchow's Arch., Bd. 28.

[17] In regard to the literature on enzymes in the urine see Huppert-Neubauer, p. 599.

Substances similar to mucin (nucleoalbumin ?) from the urinary passages and the bladder are generally present in the urine, though in very small quantities. According to several investigators normal human urine also contains traces of *proteid.*

Ptomaines and *leucomaines* or poisonous substances of an unknown kind, which are often described as alkaloidal substances, occur in normal urine (Pouchet, Bouchard, Aducco, and others). Under pathological conditions the quantity of these substances may be increased (Bouchard, Lépine and Guerin, Villiers, and others). Within the last few years the poisonous properties of urine have been the subject of more thorough investigation, especially by Bouchard. He found that the night urine is less poisonous than the day urine, and that the poisonous constituents of the day and night urines have not the same action. In order to be able to compare the toxidity of the urine under different conditions, Bouchard determines the urotoxic coefficient, which is the weight of rabbit in kilos which is killed by the quantity of urine excreted by one kilo of the person experimented upon in 24 hours.[1]

Baumann and v. Udránszky[2] have shown that ptomaines may occur in the urine under pathological conditions. They demonstrated the presence of the two ptomaines discovered and first isolated by Brieger—*putrescine*, $C_4H_{12}N_2$ (tetramethylendiamin), and *cadaverin*, $C_5H_{14}N_2$ (pentamethylendiamin)—in the urine of a patient suffering from cystinuria and catarrh of the bladder. Cadaverin has later been found by Stadthagen and Brieger[3] in the urine in two cases of cystinuria.

Brieger, v. Udránszky and Baumann, and Stadthagen have shown that not only these but other diamins occur under physiological conditions. The occurrence in normal urine of any "*urine poison*" is denied by certain investigators, such as Stadthagen.[4] The poisonous action of the urine, according to them, is due in part to the potassium salts and in part to the sum of the toxidity of the other normal urinary constituents (urea, creatinin, etc.), which have very little poisonous action individually.

Many substances have been observed in animal urine which are not found in human urine. To these belong: *kynurenic acid*, $C_{10}H_7NO_3$, which is an oxychinolincarbonic acid, occurring in dog's urine; *urocanic acid*, found in dog's urine; *damaluric acid* and *damolic acid* (according to Schotten[5] probably a mixture of benzoic acid with volatile fatty acids), obtained by the distillation of cow's urine; and lastly *lithuric acid*, found in the urinary concrements of certain animals.

III. Inorganic Constituents of Urine.

Chlorides. The chlorine occurring in urine is undoubtedly combined with the bases contained in this excretion; the chief part is combined with sodium. In accordance with this, the quantity of chlorine in the urine is generally expressed as NaCl.

The quantity of chlorine combinations in the urine is subject to considerable variation. In general the quantity for a healthy adult on a mixed diet is 10–15 grms. NaCl per 24 hours. The quantity of common salt in the urine depends chiefly upon the quantity of salt in the food, with which the elimination of chlorine increases and decreases. The free drinking of water also increases the elimination of chlorine, which is greater during activity than during rest (at night). Certain organic chlorine combinations, such as chloroform, may increase the elimination of inorganic chlorides by the urine (ZELLER,[1] MYLIUS, KAST[2]).

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[1] A complete bibliography on ptomaines and leucomaines in the urine is found in Huppert-Neubauer, p. 403. See also Griffiths, Compt. rend., Tomes 113, 114, and 115, on ptomaines in the urine in different infectious diseases.

[2] Zeitschr. f. physiol. Chem., Bd. 13.

[3] Virchow's Arch., Bd. 115.

[4] Zeitschr. f. klin. Med., Bd. 15.

[5] Zeitschr. f. physiol. Chem., Bd. 7.

In diarrhœa, in quick formation of large transudations and exudations, also in specially-marked cases of acute febrile diseases at the time of the crisis, the elimination of common salt is materially decreased. The elimination is abnormally increased in the first days after the crisis and during the absorption of extensive exudations. A diminished elimination of chlorine is found in disturbed absorption in the stomach and intestine, and in acute and chronic diseases of the kidneys accompanied with albuminuria. In chronic diseases the elimination of chlorine in general keeps pace with the nutritive condition of the body and the activity of the secretion of the urine. As under physiological conditions the quantity of common salt taken with the food has the greatest influence on the elimination of NaCl in disease

The *quantitative estimation of chlorine* in urine is most simply performed by titration with silver-nitrate solution. The urine must not contain either proteid (which if present must be removed by coagulation) or iodine or bromine compounds.

In the presence of bromides or iodides evaporate a measured quantity of the urine to dryness, fuse the residue with saltpetre and soda, dissolve the fused mass in water, and remove the iodine or bromine by the addition of dilute sulphuric acid and some nitrite, and thoroughly shake with carbon disulphide. The liquid thus obtained may now be titrated with silver nitrate according to VOLHARD'S method. The quantity of bromide or iodide is calculated as the difference between the quantity of silver-nitrate solution used for the titration of the solution of the fused mass and the quantity used for the corresponding volume of the original urine.

The otherwise excellent titration method of MOHR, according to which we titrate with silver nitrate in neutral liquids, using neutral potassium chromate as an indicator, cannot be used directly on the urine in careful work. Organic urinary constituents are also precipitated by the silver-salt, and the results are therefore somewhat high for the chlorine. If we wish to use this method, the organic urinary constituents must first be destroyed. For this purpose evaporate to dryness 5–10 c. c. of the urine, after the addition of 1 grm. of chlorine-free soda and 1–2 grms. chlorine-free saltpetre, and carefully fuse. The mass is dissolved in water, acidified faintly with nitric acid, and then neutralized exactly with pure lime carbonate. This neutral solution is used for the titration.

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[1] Zeitschr. f. physiol. Chem., Bd. 8.

[2] *Ibid.*, Bd. 11.

The silver-nitrate solution may be a $\frac{N}{10}$ solution. It is often made of such a strength that each c. c. corresponds to 0.006 grm. Cl or 0.01 grm. NaCl. This last-mentioned solution contains 29.075 grms. $AgNO_3$ in 1 litre.

Freund and Toepffer[1] have modified this method in that they titrate with silver nitrate in acetic-acid solution, which prevents the precipitation of the silver combinations of uric acid, xanthin bases, etc. Dilute 5 or 10 c. c. of the urine with 25 c. c. water, add 2.5 c. c. of a solution of acetic acid and sodium acetate (3% acid and 10% sodium acetate), and titrate after the addition of potassium chromate. Another modification has recently been suggested by Bodtker.[2]

Volhard's Method. Instead of the preceding determination, Volhard's method, which can be performed directly on the urine, may be employed. The principle is as follows: All the chlorine from the urine acidified with nitric acid is precipitated by an excess of silver nitrate, filtered, and in a measured part the quantity of silver added in excess is determined by means of a sulphocyanide solution. This excess of silver is completely precipitated by the sulphocyanide and a solution of some ferric salt, which, as is well known, gives a blood-red reaction with the smallest quantity of sulphocyanide, is used as an indicator.

We require the following solutions for this titration: 1. A silver-nitrate solution which contains 29.075 grms. $AgNO_3$ per litre and of which each c. c. corresponds to 0.01 grm. NaCl or 0.00607 grm. Cl; 2. A saturated solution at the ordinary temperature of chlorine-free iron alum or ferric sulphate; 3. Chlorine-free nitric acid of a specific gravity of 1.2; 4. A potassium-sulphocyanide solution which contains 8.3 grms. KCNS per litre, and of which 2 c. c. corresponds to 1 c. c. of the silver-nitrate solution.

About 9 grms. of potassium sulphocyanide are dissolved in water and diluted to one litre. The quantity of KCNS contained in this solution is determined by the silver-nitrate solution in the following way: Measure exactly 10 c c of the silver solution and treat with 5 c. c. of nitric acid and 1–2 c. c. of the ferric-salt solution, and dilute with water to about 100 c. c. Now the sulphocyanide solution is added from a burette, constantly stirring, until a permanent faint red coloration of the liquid takes place. The quantity of sulphocyanide found in the solution by this means indicates how much it must be diluted to be of the proper strength. Titrate once more with 10 c c. $AgNO_3$ solution and correct the sulphocyanide solution by the careful addition of water until 20 c. c. exactly correspond to 10 c. c. of the silver solution.

The determination of the chlorine in the urine is performed by this method in the following way: Exactly 10 c. c. of the urine are placed in a flask which has a mark corresponding to 100 c. c.; 5 c. c. nitric acid are added; dilute with about 50 c. c. water, and then allow exactly 20 c. c. of the silver-nitrate solution to flow in. Close the flask with the thumb and shake well, slide off the thumb and wash it with distilled water into the flask, and fill the flask to the 100-c. c. mark with distilled water. Close again with the thumb, carefully mix by shaking, and filter through a dry filter. Measure off 50 c. c. of the filtrate by means of a dry pipette, add 3 c. c. ferric-salt solution, and allow the sulphocyanide solution to flow in until the liquid above the precipitate has a permanent red color. The calculation is very simple. For example, if 4.6 c. c. of the sulphocyanide solution were necessary to produce the final reaction, then for 100 c. c. of the filtrate (= 10 c. c. urine) 9.2 c. c. of this solution are necessary. 9.2 c.c. of the sulphocyanide solution corresponds to 4.6 c. c. of the silver solution, and since $20 - 4.6 = 15.4$ c. c. of the silver solution were necessary to completely precipitate the chlorides in 10 c. c. of the urine, then 10 c. c. contain 0.154 grm. NaCl. The quantity of sodium chloride in the urine is therefore 1.54% or $15.4\,^{0}/_{00}$. If we always use 10 c. c. for the determination, and always 20 c. c. $AgNO_3$, and dilute with water to 100 c. c., we find the quantity of NaCl in 1000 parts of the urine by subtracting the number of c. c. of sulphocyanide (R) required with 50 c. c. of the filtrate from 20. The quantity of NaCl p. m. is therefore under these circumstances $= 20 - R$, and the percentage of NaCl $= \dfrac{20 - R}{10}$.

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[1] Centralbl. f. klin. Med., Bd. 13, No. 38. Cited from Maly's Jahresber., Bd. 22, S. 225.

[2] Zeitschr. f. physiol. Chem., Bd. 20.

The approximate estimation of chlorine in the urine (which must be free from proteid) is made by strongly acidifying with nitric acid and then adding to it, drop by drop, a concentrated silver-nitrate solution (1 : 8). In a normal quantity of chlorides the drop sinks to the bottom as a rather compact cheesy lump. In diminished quantities of chlorides the precipitate is less compact and coherent, and in the presence of very little chlorine a fine white precipitate or only a cloudiness or opalescence is obtained.

Phosphates. Phosphoric acid occurs in acid urines partly as double-, MH_2PO_4, and partly as simple-acid, M_2HPO_4, phosphates, both of which are found in acid urines at the same time. OTT[1] found that on an average 60% of the total phosphoric acid was double- and 40% was simple-acid phosphate. The total quantity of phosphoric acid is very variable and depends on the kind and the quantity of food. The average quantity of P_2O_5 is in round numbers 2.5 grms., with a variation of 1–5 grms., per 24 hours. A small part of the phosphoric acid of the urine originates from the burning of organic compounds, nuclein, protagon, and lecithin, within the organism. The greater part originates from the phosphates of the food, and the quantity of eliminated phosphoric acid is greater when the food is rich in alkali phosphates in proportion to the quantity of lime and magnesia phosphates. If the food contains much lime and magnesia, large quantities of earthy phosphates are eliminated by the excrements; and even though the food contains considerable amounts of phosphoric acid in these cases, the quantity of phosphoric acid in the urine is small. Such a condition is found in herbivora, whose urine is habitually poor in phosphates. The extent of the elimination of phosphoric acid by the urine depends not only upon the total quantity of phosphoric acid in the food, but also upon the relative amounts of alkaline earths and the alkali salts in the food. According to PREYSZ,[1] OLSAVSKY and KLUG[2] the elimination of phosphoric acid is considerably increased by intense muscular work.

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[1] Zeitschr. f. physiol. Chem., Bd. 10.

From the transformation of tissues rich in proteid or of phosphorized nerve-substance in the body we might perhaps expect an equal relation between the nitrogen and the phosphoric acid in the urine. Many investigations have been made upon this subject, but as all the conditions which affect the elimination of phosphoric acid are not yet sufficiently known, it is difficult to draw any definite conclusions from the observations thus far made.

As the extent of the elimination of phosphoric acid is mostly dependent upon the character of the food and the absorption of the phosphates in the intestine, it is apparent that the relationship between the nitrogen and phosphoric acid in the urine can only be approximately constant with a certain uniform food. Thus, on feeding with exclusive meat diet, as observed by VOIT[3] on dogs, when the nitrogen and phosphoric acid (P_2O_5) of the food exactly reappeared in the urine and fæces the relationship was 8.1 : 1. In starvation this relationship is changed, namely, relatively more phosphoric acid is eliminated, which seems to indicate that besides flesh and related tissues also another tissue rich in phosphorus is largely destroyed. The starvation experiments show that this tissue is the bone tissue.

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[1] See Maly's Jahresber., Bd. 21.

[2] Pflüger's Arch., Bd. 54.

[3] Physiologie des allgemeinen Stoffwechsels und der Ernährung in L. Hermann's Handbuch, Bd. 6, Thl. 1, S. 79.

Little is known in regard to the elimination of phosphoric acid in disease. In febrile diseases, as shown by several observations, the quantity of phosphoric acid as compared with the nitrogen is considerably decreased. In diseases of the kidneys the activity of these organs in eliminating the phosphates may be considerably diminished (FLEISCHER[1]). In meningitis, on the contrary, a marked increase in the phosphates is observed in the urine. TEISSIÈR has described a special form of polyuria, in which large quantities of earthy phosphates, 10–20–30 grms. per 24 hours, were eliminated. This polyuria was called PHOSPHATE DIABETES[2] by TEISSIÈR. The statements in regard to the quantity of phosphate in the urine in rachitis and in osteomalacia are somewhat contradictory.[3]

Quantitative estimation of phosphoric acid in the urine. This estimation is most simply performed by titrating with a solution of uranium acetate. The principle of the titration is as follows: A warm solution of phosphates containing free acetic acid gives a whitish-yellow precipitate of uranium phosphate with a solution of a uranium salt. This precipitate is insoluble in acetic acid, but dissolves in mineral acids, and on this account we always add in titrating a certain quantity of sodium-acetate solution. Potassium ferrocyanide is used as the indicator, which does not act on the uranium-phosphate precipitate, but gives a reddish-brown precipitate or coloration in the presence of the smallest amount of soluble uranium salt. The solutions necessary for the titration are: 1. A solution of a uranium salt of which each c. c. corresponds to 0.005 grm. P_2O_5 and which contains 20.3 grms. uranium oxide per litre. 20 c. c. of this solution corresponds to 0.100 grm. P_2O_5. 2. A solution of sodium acetate; 3. A freshly prepared solution of potassium ferrocyanide.

The uranium solution is prepared from uranium nitrate or acetate. Dissolve about 35 grms. uranium acetate in water, add some acetic acid to facilitate solution, and dilute to one litre. The strength of this solution is determined by titrating with a solution of sodium phosphate of known strength (10.085 grms crystallized salt in 1 litre, which corresponds to 0.100 grm. P_2O_5 in 50 c. c.). Proceed in the same way as in the titration of the urine (see below), and correct the solution by diluting with water, and titrate again until 20 c. c. of the uranium solution corresponds exactly to 50 c. c. of the above phosphate solution.

The sodium-acetate solution should contain 10 grms. sodium acetate and 10 grms. conc. acetic acid in 100 c. c. For each titration 5 c. c. of this solution is used with 50 c. c. of the urine.

In performing the titration, mix 50 c. c. of filtered urine in a beaker with 5 c. c. of the sodium acetate, cover the beaker with a watch-glass, and warm over the water-bath. Then allow the uranium solution to flow in from a burette, and, when the precipitate does not seem to increase, place a drop of the mixture on a porcelain plate with a drop of the potassium-ferrocyanide solution. If the amount of uranium solution employed is not sufficient, the color remains pale yellow and more uranium solution must be added; but as soon as the slightest excess of uranium solution has been used, the color becomes faint reddish brown. When this point has been obtained, warm the solution again and add another drop. If the color remains of the same intensity, the titration is ended; but if the color varies, add more uranium solution, drop by drop, until a permanent coloration is obtained after warming, and now repeat the test with another 50 c. c. of the urine. The calculation is so simple that it is unnecessary to give an example.

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[1] Deutsch. Arch. f. klin. Med., Bd. 29.

[2] Centralbl. f. d. med. Wissensch., 1877.

[3] In regard to the elimination of phosphates in disease see Neubauer-Huppert-Thomas, Harnanalyse, 9. Aufl., Semiotischer Theil, S. 255–267.

In the above manner we determine the total quantity of phosphoric acid in the urine. If we wish to know the phosphoric acid combined with alkaline earths or with alkalies, we first determine the total phosphoric acid in a portion of the urine and then remove the earthy phosphates in another portion by ammonia. The precipitate is collected on a filter, washed, transferred in a beaker with water, treated with acetic acid, and dissolved by warming. This solution is now diluted to 50 c. c. with water, and 5 c. c. sodium-acetate solution added, and titrated with uranium solution. The difference between the two determinations gives the quantity of phosphoric acid combined with the alkalies. The results obtained are not quite accurate, as a partial transformation of the monophosphates of the alkaline earths and also calcium diphosphate into triphosphates of the alkaline earths and ammonium phosphate takes place on precipitating with ammonia, which gives too high results for the phosphoric acid combined with alkalies remaining in solution.

Sulphates. The sulphuric acid of the urine originates only to a very small extent from the sulphates of the food. A disproportionally greater part is formed by the burning of the proteids containing sulphur within the body, and it is chiefly this formation of sulphuric acid from the proteids which gives rise to the previously mentioned excess of acids over the bases in the urine. The quantity of sulphuric acid eliminated by the urine amounts to about 2.5 grms. H_2SO_4 per twenty-four hours. As the sulphuric acid chiefly originates from the proteids, it follows that the elimination of sulphuric acid and the elimination of nitrogen are nearly parallel, and the relationship $N : H_2SO_4$ is about 5 : 1. A complete parallelism can hardly be expected, as in the first place a part of the sulphur is always eliminated as neutral sulphur, and secondly because the low quantity of sulphur in different protein bodies undergoes greater variation as compared with the high quantity of nitrogen contained therein. Generally the relationship between the elimination of nitrogen and sulphuric acid, under normal and diseased conditions, runs rather parallel. Sulphuric acid occurs in the urine partly preformed (sulphate-sulphuric acid) and partly as ethereal-sulphuric acid. The first is designated as *A*- and the other as *B*-sulphuric acid.

The *quantity of total sulphuric acid* is determined in the following way, but at the same time the precautions described in other works must be observed: 100 c. c. of filtered urine are treated with 5 c. c. concentrated hydrochloric acid and boiled for fifteen minutes. While boiling precipitate with 2 c. c. of a saturated $BaCl_2$ solution and warm for a little while until the barium sulphate has completely settled. The precipitate must then be washed with water and also with alcohol and ether (to remove resinous substances) and then treated according to the usual method.

The separate determination of the sulphate-sulphuric acid and the ethereal-sulphuric acid may be accomplished, according to BAUMANN's[1] method, by first precipitating the sulphate-sulphuric acid from the urine acidified with acetic acid by $BaCl_2$, and then decomposing the ethereal-sulphuric acid by boiling after the addition of hydrochloric acid, and then determining the sulphuric acid set free as barium sulphate. A still better method is the following suggested by SALKOWSKI[2]:

200 c. c. of urine are precipitated by an equal volume of a barium solution which consists of 2 vols. barium hydrate and 1 vol. barium-chloride solution, both saturated at the ordinary temperature. Filter through a dry filter, measure off 100 c. c. of the filtrate which contains only the ethereal-sulphuric acid, treat with 10 c. c. hydrochloric acid of a specific gravity 1.12, boil for fifteen minutes, and then warm on the water-bath until the precipitate has completely settled and the supernatant liquid is entirely clear. Filter and wash with warm water and with alcohol and ether and proceed according to the generally prescribed method. The difference between the ethereal-sulphuric acid found and the total quantity of sulphuric acid as determined in a special portion of urine is considered as the quantity of sulphate-sulphuric acid.

Nitrates occur in small quantities in human urine (**SCHÖNBEIN**[3]), and they probably originate from the drinking-water and the food. According to WEYL and CITRON,[4] the quantity of nitrates is smallest with a meat diet and greatest with vegetable food. The average amount is about 42.5 milligrammes per litre.

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[1] Zeitschr. f. physiol. Chem., Bd 1, S. 70.

[2] Virchow's Arch., Bd. 79.

[3] Journ. f. prakt. Chem., Bd. 92, S. 152.

[4] Virchow's Arch., Bdd. 96 u. 101.

Potassium and Sodium. The quantity of these bodies eliminated by the urine by a healthy full-grown person on a mixed diet is, according to SALKOWSKI,[1] 3–4 grms. K_2O and 5–8 grms. Na_2O, with an average of about 2–3 grms. K_2O and 4–6 grms. Na_2O. The proportion of K to Na is ordinarily as 3 : 5. The quantity depends above all upon the food. In starvation the urine may become richer in potassium than in sodium, which results from the lack of common salt and the destruction of tissue rich in potassium. The quantity of potassium may be relatively increased during fever, while after the crisis the reverse is the case.

The quantitative estimation of these bodies is performed by the gravimetric methods as described in works on quantitative analysis.

Ammonia. Some ammonia is habitually found in human urine and in that of carnivora. This ammonia may represent, as above stated (page 455), on the formation of urea from ammonia, the small amount of ammonia which, because of the excess of acids formed by the combustion, as compared with the fixed alkalies, is united with such acids, and in this way is excluded from the synthesis to urea. This view is confirmed by the observations of CORANDA,[2] who found that the elimination of ammonia was smaller on a vegetable diet and larger on a rich meat diet than when on a mixed diet. On a mixed diet the average amount of ammonia eliminated by the urine is about 0.7 grm. NH_3 per twenty-four hours (NEUBAUER[3]). All the ammonia of the urine, as above stated, is not represented by the residue which has eluded synthesis into urea by neutralization by acids because, as shown by STADELMANN and BECKMANN,[4] ammonia is eliminated by the urine even during the continuous administration of fixed alkalies.

The experiments of many investigators[5] have shown that in man and carnivora no formation of urea takes place from ammonia salts with mineral acids such as ammonium chloride, but they are eliminated as such in the urine, while, on the contrary, in herbivora a formation of urea may take place from ammonium chloride. In herbivora the HCl of the ammonium chloride combines with fixed alkalies, and the ammonia set free is available for the formation of urea. This difference in the behavior of ammonium chloride in carnivora and herbivora is dependent upon the different behavior of the acids in the organism of these two groups of animals. The quantity of ammonia in human and carnivoral urine is increased by the introduction of mineral acids, and, as shown by JOLIN,[1] organic acids, like benzoic acid, which is not burned in the body, act in a similar way. This depends upon the fact that the organism of these animals has the property of producing sufficient ammonia by destruction of proteids to neutralize the acids introduced and in this way prevent a destructive abstraction of fixed alkalies. Herbivora, on the contrary, lack this property. In them the acids introduced are neutralized by fixed alkalies; hence the introduction of mineral acids soon causes a destructive action on account of the abstraction of alkalies.

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[1] Virchow's Arch., Bd. 53.

[2] Arch. f. exp. Path. u. Pharm., Bd. 12.

[3] Huppert Neubauer, Harnanalyse, 10. Aufl., S. 42.

[4] Stadelmann, Einfluss der Alkalien auf den Stoffwechsel des Menschen. Stuttgart, 1890, S. 52.

[5] See footnotes page 455.

Acids formed in the destruction of proteids in the body act like those introduced from without on the elimination of ammonia. For this reason the quantity of ammonia in human and carnivoral urine is increased under such conditions and in such diseases where an increased formation of acid takes place due to an increased metabolism of proteids. This is the case in fevers and diabetes. In the last-mentioned disease an organic acid, β-oxybutyric acid, is produced, which passes into the urine combined with ammonia. As the elimination of ammonia and the formation of urea stand in close relation to each other, it was expected that an increase in the elimination of ammonia and a decrease in the formation of urea would take place in certain diseases of the liver. We have given above, on the formation of urea in the liver, the extent of agreement of this statement, and the reader is referred to the works there cited.

The detection and quantitative estimation of ammonia is performed generally according to the method suggested by SCHLÖSING. The principle of this method is that the ammonia from a measured amount of urine is set free by lime-water in a closed vessel and absorbed by a measured amount of $\frac{N}{10}$ sulphuric acid. After the absorption of the ammonia the quantity is determined by titrating the remaining free sulphuric acid with a $\frac{N}{10}$ caustic alkali. This method gives low results, and in exact work we must proceed as suggested by Bohland.[1] Other methods have been suggested by Schmiedeberg[2] and by Latschenberger.[3]

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[1] Skand. Arch. f. Physiol., Bd. 1.

Calcium and **magnesium** occur in the urine for the most part as phosphates. The quantity of earthy phosphates eliminated daily is somewhat more than 1 gr., and of this amount $\frac{2}{3}$ is magnesium and $\frac{1}{3}$ calcium phosphate. In acid urines the simple- as well as the double-acid earthy phosphates are found, and the solubility of the first, among which the calcium-salt, $CaHPO_4$, is especially insoluble, is particularly augmented by the presence of double-acid alkali phosphate and sodium chloride in the urine (Ott[4]). The quantity of alkaline earths in the urine depends on the composition of the food. Nothing is known with positiveness in regard to the constant and regular change in the elimination of these substances in disease.

The quantity of calcium and magnesium is determined according to the ordinary well-known methods.

Iron occurs in the urine only in small quantities, and, as it seems from the investigations of Kunkel,[5] Giacosa,[6] Kobert,[7] and his pupils, it does not exist as a salt, but as an organic combination—in part as pigment or chromogen. The statements in regard to the quantity of iron seem to show that the quantity is very variable, from 1 to 11 milligrammes per litre of urine (Magnier,[8] Gottlieb,[9] Kobert, and his pupils). The quantity of *silicic acid*, according to the ordinary statements, amounts to about 0.03 p. m. Traces of *hydrogen peroxide* also occur in the urine.

The *gases* of the urine are carbon dioxide, nitrogen, and traces of oxygen. The quantity of nitrogen is not quite 1 vol. per cent. The carbon dioxide varies considerably. In acid urines it is hardly one half as great as in neutral or alkaline urines.

IV. The Quantity and Quantitative Composition of Urine.

A direct participation of the kidney substance in the formation of the urinary constituents is proved at least for one constituent of the urine, namely, hippuric acid. It is hardly to be doubted that the kidneys as well as the tissues generally have a certain part to play in the formation of other urinary constituents, but their chief task consists in separating and removing urinary constituents dissolved in the blood which have been taken up by it from other organs and tissues.

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[1] Pflüger's Arch., Bd. 43.

[2] Arch. f. exp. Path. u. Pharm., Bd. 7.

[3] Monatshefte f. Chem., Bd. 5.

[4] Zeitschr. f. physiol. Chem., Bd. 10.

[5] Sitzungsber. d. phys.-med. Gesellsch. zu Würzburg, 1881. Cited from Maly's Jahresber., Bd. 11, S. 246.

[6] See Maly's Jahresber., Bd. 16, S. 213.

[7] Arbeiten des pharm. Instit. zu Dorpat, Bd. 7. Stuttgart, 1891.

[8] Ber. d. deutsch. chem. Gesellsch., Bd. 7.

[9] Arch. f. exp. Path. u. Pharm., Bd. 26.

It has been shown by the experiments of numerous investigators, HEIDENHAIN, v. WITTICH, NUSSBAUM, NEISSER, USTIMOWITSCH, I. MUNK, and others, that the elimination of water and the remaining urinary constituents is not alone produced by simple diffusion and filtration.[1] It is generally conceded that the processes of urinary secretion depend essentially upon a specific activity of the cells of the epithelium of the urinary passages, besides which also processes of filtration and diffusion take part. The process of the secretion of urine in man and the higher animals is generally considered to proceed chiefly as follows: The water together with a small amount of the salts passes through the glomeruli, while the chief part of the solids is secreted by the epithelium of the urinary passages. A secretion of solids without a simultaneous secretion of water is not possible, and therefore a part of the water must be secreted by the epithelium-cells of the urinary passages. The passage of the chief part of the water through the glomeruli is rather generally considered as a filtration due to blood-pressure. According to HEIDENHAIN, the thin cell-layers of the glomeruli have a secretory action.

The quantity and the composition of urine are liable to great variation. Those circumstances which under physiological conditions exercise a great influence are the following: the blood-pressure, and the rapidity of the blood-current in the glomeruli; the quantity of urinary constituents, especially water in the blood; and lastly, the condition of the secretory glandular elements. Above all, the quantity and concentration of the urine depend on the elimination of water. That this last may vary with the quantity of water in the blood, with changed blood-pressure, and with circulatory conditions is evident; but under ordinary circumstances the quantity of water eliminated by the kidneys depends essentially upon the quantity of water which is brought to them by the blood or which leaves the body by other exits. The elimination of urine is increased by drinking freely, or by reducing the quantity of water removed in other ways; but it is decreased by a diminished introduction of water, or by a greater loss of water in other ways. Ordinarily in man just as much water is eliminated by the kidneys as by the skin, lungs, and intestine together. At lower temperatures and in moist air, since under these conditions the elimination of water by the skin is diminished, the elimination of urine may be considerably increased. Diminished introduction of water or increased elimination of water by other means—as in violent diarrhœa, violent vomiting, or abundant perspiration—greatly diminishes the elimination of urine. For example, the urine may sink as low as 500–400 c. c. per day in intense summer-heat, while after copious draughts of water the elimination of 3000 c. c. of urine has been observed during the same time. The average quantity of urine voided in the course of 24 hours must undergo considerable variation; ordinarily it is calculated as 1500 c. c. for healthy adult men and 1200 c. c. for women. The minimum elimination occurs during the night, between 2 and 4 o'clock; the maximum, in the first hours after awaking and from 1–2 hours after a meal.

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[1] See Heidenhain, Die Harnabsonderung in Hermann's Handbuch, Bd. 5, Thl. 1, S. 279.

The quantity of solids excreted in the course of 24 hours is rather constant even though the quantity of urine may vary, and it is more constant when the manner of living is regular. Therefore the percentage of solids in the urine is naturally in an inverse proportion to the quantity of urine. The average quantity of solids per 24 hours is calculated as 60 grms. The quantity may be calculated with approximate accuracy by means of the specific gravity if the second and third decimals of the specific gravity be multiplied by HÄSER's coefficient, 2.33. The product gives the amount of solids in 1000 c. c. of urine, and if the quantity of urine eliminated in the 24 hours be measured, the quantity of solids in the 24 hours may be easily calculated. For example, 1050 c. c. of urine of a specific gravity 1.021 was eliminated in the 24 hours; therefore the quantity of solids eliminated is $21 \times 2.33 = 48.9$, and $\frac{48.9 \times 1050}{1000} = 51.35$ grms. The urine in this case contained 48.9 p. m. solids and 51.35 grms. in the daily excretion.

Those bodies which, under physiological conditions, affect the density of the urine are common salt and urea. The specific gravity of the first is 2.15 and the last only 1.32, so it is easy to understand, when the relative proportion of these two bodies essentially deviates from the normal, why the above calculation from the specific gravity is not exact. The same is the case when a urine poor in a normal constituent contains large amounts of foreign bodies, such as albumin or sugar.

As above stated, the percentage of solids in the urine generally decreases with a greater elimination, and a very considerable excretion of urine (*polyuria*) has therefore, as a rule, a lower specific gravity. An important exception to this rule is observed in urine containing sugar (*diabetes mellitus*), in which there is a copious excretion of a very high specific gravity due to the sugar. In cases where very little urine is secreted (*oliguria*), as when the perspiration is profuse, in diarrhœa, and in fevers, the specific gravity of the urine is as a rule high, the percentage of solids high, and has a dark color. Sometimes, as, for example, in certain cases of albuminuria, the urine may have a low specific gravity, notwithstanding the oliguria, and be poor in solids with a light color.

It is difficult to give a tabular view of the composition of urine, on account of its variation. For certain purposes the following table may be of some value, but it must not be overlooked that the results are not given for 1000 parts of urine, but only approximate figures for the quantities of the most important constituents which are eliminated in the course of 24 hours in a quantity of 1500 c. c.

Daily quantity of solids = 60 grms.

Organic constituents = 35 grms.		Inorganic constituents = 25 grms.	
Urea	30.0 grms.	Sodium chloride ($NaCl$)	15.0 grms.
Uric acid	0.7 "	Sulphuric acid (H_2SO_4)	2.5 "
Creatinin	1.0 "	Phosphoric acid (P_2O_5)	2.5 "
Hippuric acid	0.7 "	Potash (K_2O)	3.3 "
Remaining organic bodies	2.6 "	Ammonia (NH_3)	0.7 "
		Magnesia (MgO)	0.5 "
		Lime (CaO)	0.3 "
		Remaining inorg. bodies	0.2 "

Urine contains on an average 40 p. m. solids. The quantity of urea is about 20 p. m. and common salt about 10 p. m.

V. Casual Urinary Constituents.

The casual appearance in the urine of medicines or of urinary constituents resulting from the introduction of foreign substances into the organism is of practical importance, because such constituents may interfere in certain urinary investigations, and also because they afford a good means of determining whether certain substances have been introduced into the organism or not. From this point of view a few of these bodies will be spoken of in a following section (on the pathological urinary constituents). The presence of these foreign bodies in the urine is of special interest in those cases in which they serve to elucidate the chemical transformations certain substances undergo within the body. As inorganic substances generally leave the body unchanged, they are of very little interest from this standpoint, but the changes which certain organic substances undergo when introduced into the animal body may be studied by this means so far as these transformations are shown by the urine.

The bodies belonging to the **fatty series**, though not without exceptions, fall mostly into a combustion leading towards the final products of metabolism; still, often a smaller or greater part of the body in question eludes oxidation and appears unchanged in the urine. A part of the organic acids, which are otherwise burnt into water and carbonates and render the urine neutral or alkaline, may act in this manner. The *volatile fatty acids* poor in carbon are less easily burnt than those rich in carbon, and they therefore pass unchanged into the urine in large amounts. This is especially true of formic and acetic acids (SCHOTTEN,[1] GRÉHANT and QUINQUAUD[2]). According to GAGLIO *oxalic acid* is not oxidized in the animal body, while MARFORI[3] claims that it is nearly entirely consumed.

The *acid amides* appear not to be changed in the body (SCHULTZEN and NENCKI[4]). A small part of the *amido-acids* seems indeed to be eliminated unchanged, but otherwise they are, as stated above (page 455) for *leucin*, *glycocoll*, and *aspartic acid*, decomposed within the body, and they may therefore cause an increased elimination of urea. *Sarcosin* (methylglycocoll), $NH(CH_3).CH_2.COOH$, also perhaps passes in small part into the corresponding uramido-acid, *methylhydantoinic* acid, $NH_2.CO.N(CH_3).CH_2.COOH$ (SCHULTZEN[5]). Also *taurin*, amido-ethylsulphonic acid, which acts somewhat differently in different animals (SALKOWSKI[6]), passes in human beings, at least in part, into the corresponding uramido-acid, *taurocarbamic acid*, $NH_2.CO.NH.C_2H_4.SO_2.OH$. A part of that certain amido-acids, such as *tyrosin*, *phenylamido-propionic acid*, and *amido-cinnamic acid* when introduced into the body cause no increase in the quantity of known aromatic substances in the urine; this makes a destruction of these amido-acids in the animal body seem probable. Juvalta[1] also made an experiment on dogs with *phthalic acid*, and found that 57.5–68.76% of the acid introduced into the body disappeared, or more correctly was not found again. According to Juvalta, this acid does not undergo any synthesis, nor does it yield any aromatic transformation products; and if this supposition be correct, we have here a proof of the destruction of the benzol nucleus of a part of the phthalic acid introduced into the organism of the dog.

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[1] Zeitschr. f. physiol. Chem., Bd. 7, S. 375.

[2] Compt. rend., Tome 104.

[3] See Maly's Jahresber., Bd. 16, S. 402, and Bd. 20, S. 70.

[4] Zeitschr. f. Biologie, Bd. 8.

[5] Ber. d. deutsch. chem. Gesellsch., Bd. 5. See also Baumann and v. Mering, *ibid.*, Bd. 8, S. 584, and E. Salkowski, Zeitschr. f. physiol. Chem., Bd. 4, S. 107.

[6] Ber. d. deutsch. chem. Gesellsch., Bd. 6, and Virchow's Arch., Bd. 58.

An *oxidation* in the side chain of aromatic compounds is often found, and may also occur in the nucleus itself. As an example, benzol is first oxidized to oxybenzol (Schultzen and Naunyn[2]), and this is then in part converted into *dioxybenzols* (Baumann and Preusse[3]). *Naphthalin* appears to be converted into *oxynaphthalin*, and probably a part also into *dioxynaphthalin* (Lesnik and M. Nencki[4]). Anilin, $C_6H_5.NH_2$, passes into paramidophenol,[5] which passes into the urine as ethereal-sulphuric acid, $H_2N.C_6H_4.O.SO_2.OH$ (F. Müller[6]).

If the aromatic substance has a side chain belonging to the fatty series, this last is generally oxidized. For example, *toluol*, $C_6H_5.CH_3$ (Schultzen and Naunyn[7]), *ethyl-benzol*, $C_6H_5.C_2H_5$, and *propylbenzol*, $C_6H_5.C_3H_7$ (Nencki and Giacosa[8]), also many other bodies are oxidized into benzoic acid. If the side chain has several members, the behavior is somewhat different. *Phenyl-acetic acid*, $C_6H_5.CH_2.COOH$, in which only one carbon atom exists between the benzol nucleus and the carboxyl, is not oxidized, but

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Zeitschr. f. physiol. Chem., Bd. 6; Schotten, *ibid.*, Bd. 7; Baas, *ibid.*, Bd. 11; and R. Cohn, *ibid.*, Bd. 14.

[1] Zeitschr. f. physiol. Chem., Bd. 13.

[2] Reichert's und Du Bois-Reymond's Arch., 1867.

[3] Zeitschr. f. physiol. Chem., Bd. 3, S. 156. See also Nencki and Giacosa, *ibid.*, Bd. 4, S. 336.

[4] Arch. f. exp. Path. u. Pharm., Bd. 24. See also Edlefsen, Maly's Jahresber., Bd. 18, S. 116.

[5] Schmiedeberg, Arch. f. exp. Path. u. Pharm., Bd. 8.

[6] Deutsch. med. Wochenschr., 1887. Cited from Maly's Jahresber., Bd. 17, S. 87.

[7] Reichert's and Du Bois-Reymond's Arch., 1867.

[8] Zeitschr. f. physiol. Chem., Bd. 4.

is eliminated after conjugation with glycocoll as *phenaceturic acid* (Salkowski[1]). *Phenyl-propionic acid*, $C_6H_5.CH_2.CH_2.COOH$, with two carbon atoms between the benzol nucleus and the carboxyl is, on the contrary, oxidized into benzoic acid.[2] Aromatic amido-acids with three carbon atoms in the side chain, and where the NH_2 group is bound to the middle one, as in *tyrosin*, α-oxyphenylamido-propionic acid, $C_6H_4(OH).CH_2.CH(NH_2).COOH$, and *$\alpha$-phenyl-amido-propionic acid*, $C_6H_5.CH_2.CH(NH_2).COOH$, seem to be in great part burnt within the body. *Phenylamido-acetic acid*, which has only two carbon atoms in the side chain, $C_6H_5.CH(NH_2).COOH$, acts otherwise, passing into *mandelic acid*, phenyl-glycolic acid, $C_6H_5.CH(OH).COOH$ (Schotten[3]).

If several side chains are present in the benzol nucleus, then only one is always oxidized into carboxyl. Thus *xylol*, $C_6H_4(CH_3)_2$, is oxidized into *toluic acid*, $C_6H_4(CH_3)COOH$ (Schultzen and Naunyn[4]), *mesitylen*, $C_6H_3(CH_3)_3$, into *mesitylenic acid*, $C_6H_3(CH_3)_2.COOH$ (L. Nencki[5]), and *cymol* into *cumic acid* (M. Nencki and Ziegler[6]).

Syntheses of aromatic substances with other atomic groups occur frequently. To these syntheses belongs, in the first rank, the conjugation of *benzoic acid* with glycocoll to form *hippuric acid*, first discovered by Wöhler. All the numerous aromatic substances which are converted into benzoic acid in the body are voided partly as hippuric acid. This statement is not true for all classes of animals. According to the observations of Jaffé,[7] benzoic acid does not pass into hippuric acid in birds, but into another nitrogenous acid, *ornithuric acid*, $C_{19}H_{20}N_2O_4$. This acid yields as splitting products, besides benzoic acid, a body, *ornithin*, which has been spoken of on page 524. Not only are the *oxybenzoic acids* and the *substituted benzoic acids* (Bertagnini[8]) conjugated with glycocoll, forming corresponding hippuric acids, but also the above-mentioned acids, *toluic*, *mesitylenic*, *cumic*, and *phenylacetic acids*.

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[1] Zeitschr. f. physiol. Chem., Bdd. 7 and 9.

[2] See E. and H. Salkowski, Ber. d. deutsch. chem. Gesellsch., Bd. 12.

[3] Zeitschr. f. physiol. Chem., Bd. 8.

[4] Reichert's und Du Bois-Reymond's Arch., 1867.

[5] Arch. f. exp. Path. u. Pharm., Bd. 1.

[6] Ber. d. deutsch. chem. Gesellsch., Bd. 5; see also O. Jacobsen, *ibid.*, Bd. 12.

[7] *Ibid.*, Bdd. 10 and 11.

[8] Cited from Kühne's Lehrbuch, S. 91.

These acids are voided as ***toluric***, ***mesitylenuric***, ***cuminuric***, and *phenaceturic acids*.

It must be remarked in regard to the oxybenzoic acids that a conjugation with glycocoll has only been positively proven with salicylic acid and p-oxybenzoic acid (BERTAGNINI, BAUMANN and HERTER,[1] and others), while BAUMANN and HERTER find it only very probable for m-oxybenzoic acid. The oxybenzoic acids are also in part eliminated as conjugated sulphuric acids, which is especially true for m-oxybenzoic acid.[2] We have the investigations on m-amidobenzoic acid in regard to the transformation of amidobenzoic acids. SALKOWSKI[3] found, as was later confirmed by R. COHN,[4] that m-amidobenzoic acid passes in part into *uramidobenzoic* acid, $H_2N.CO.HN.C_6H_4.COOH$. It is also in part eliminated as amidohippuric acid.

The substituted aldehydes are of special interest as substances which undergo conjugation with glycocoll. According to the investigations of R. COHN[5] on this subject o-nitrobenzaldehyde when introduced into a rabbit is only in a very small part converted into nitrobenzoic acid, and the chief mass, about 90%, is destroyed in the body. According to SIEBER and SMIRNOW[6] m-nitrobenzaldehyde passes in dogs into m-nitrohippuric acid, and according to COHN into urea m-nitrohippurate. In rabbits the behavior is quite different according to COHN. In this case not only does an oxidation of the aldehyde into benzoic acid take place, but the nitro group is also reduced to an amido group, and finally acetic acid attaches itself to the amido group with the expulsion of water, so that the final product, m-acetylamidobenzoic acid, $CH_3.CO.NH.C_6H_4.COOH$, is the result. This process is analogous to the behavior of furfurol, and the reduction does not take place in the intestine, but in the tissue.[7] The p-nitrobenzaldehyde acts in rabbits in part like the m-aldehyde and passes in part into *p-acetylamidobenzoic acid*. Another part is converted into p-nitrobenzoic acid, and the urine contains a chemical combination of equal parts of these two acids. According to SIEBER and SMIRNOW p-nitrobenzaldehyde only yields urea p-nitrohippurate in dogs.[1]

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[1] Zeitschr. f. physiol. Chem., Bd. 1, which also cites Bertagnini's work.

[2] See Baumann and Herter, l. c., and also Dautzenberg in Maly's Jahresber., Bd. 11, S. 231.

[3] Zeitschr. f. physiol. Chem., Bd. 7.

[4] *Ibid.*, Bd. 17, S. 292.

[5] *Ibid.*, Bd. 17.

[6] Monatshefte. f. Chem., Bd. 8.

[7] Zeitschr. f. physiol. Chem., Bd. 18.

Another very important synthesis of aromatic substances is that of the *ethereal-sulphuric acids*. *Phenols* and chiefly the *hydroxylated aromatic hydrocarbons* and their derivatives are voided as ethereal-sulphuric acids, according to BAUMANN, HERTER, and others.[2]

A conjugation of aromatic substances with glycuronic acid, which last is protected from burning, occurs rather often. *Camphor*, $C_{10}H_{16}O$, when given to a dog is first converted by oxidation into campthoral, $C_{10}H_{15}(OH)O$, and by conjugation with glycuronic acid into *campho-glycuronic acid* (SCHMIEDEBERG[3]). The phenols, as above stated (page 491), pass in part as conjugated glycuronic acids into the urine. The same is true for the homologues of phenols, for certain substituted phenols, for *naphthols*, *borneol*, *menthol*, *turpentine*, and many other aromatic substances.[4] *Orthonitrotoluol* in dogs passes first into o-nitrobenzyl alcohol and then into a conjugated glycuronic acid, *uronitrotoluolic acid* (JAFFÉ[5]). The glycuronic acid split off from the conjugated acid is lævogyrate and hence not identical with the ordinary glycuronic acid, but isomeric. *Indol* and *skatol* seem, as above stated (page 495 and 496), to be eliminated in the urine partly as conjugated glycuronic acids.

A synthesis in which compounds containing sulphur, *mercapturic acid*, is formed and eliminated conjugated with glycuronic acid, occurs when chlorine and bromine derivates of benzol are introduced into the organism of dogs (BAUMANN and PREUSSE,[6] JAFFÉ[7]). Thus *chlorbenzol* combines with CYSTEIN, an intermediary decomposition product of proteids which is closely allied to cystin (see below), forming *chlorphenylmercapturic acid* $C_{11}H_{12}ClSNO_3$. On boiling with mineral acid this compound decomposes into acetic acid and chlorphenylcystein, $C_6H_4Cl.C_3H_6NSO_2$.

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[1] In regard to the extensive literature on glycocoll conjugations we refer the reader to O. Kühling, Ueber Stoffwechselprodukte aromatischer Körper. Inaug.-Diss. Berlin, 1887.

[2] See O. Kühling, l. c.

[3] Schmiedeberg und Meyer, Zeitschr. f. physiol. Chem., Bd. 3.

[4] See O. Kühling, l. c., which gives the literature up to 1887; also E. Külz, Zeitschr. f. Biologie, Bd. 27.

[5] Zeitschr. f. physiol. Chem., Bd. 2.

[6] *Ibid.*, Bd. 5, S. 309.

[7] Ber. d. deutsch. chem. Gesellsch., Bd. 12.

Pyridin, C_5H_5N, which does not combine either with glycuronic acid or with sulphuric acid after previous oxidation, shows a special behavior. It takes up a methyl group as found by His[1] and later confirmed by Cohn,[2] and forms an ammonium combination, *methylpyridyl-ammonium hydroxyl*, $HO.CH_3.NC_5H_5$. Methylpyridin (*α-picolin*) on the contrary passes in rabbits part in into *α-pyridin carbonic acid*, and is eliminated as *α-pyridinuric acid* after conjugation with glycuronic acid (R. Cohn[3]). Several alkaloids, such as *quinin*, *morphin*, and *strychnin*, may pass into the urine. After taking *turpentine*, *balsam of copaiva*, and *resins* these may appear in the urine as resin acids. Different kinds of coloring matters, such as *alizarin*, *crysophanic acid*, after the use of rhubarb or senna, and the *coloring matter of the blueberry*, etc., may also pass into the urine. After taking *rhubarb*, *senna*, or *santonin* the urine takes a yellow or greenish-yellow color, which is transformed into a beautiful red color by the addition of alkali. *Phenol* produces, as above mentioned, a dark-brown or dark-green color which depends mainly on the decomposition products of hydrochinon and humin substances. After the use of *naphthalin* the urine has a dark color, and several other medicines produce a special coloration. Thus *kairin* gives often a yellowish-green hue, and the urine darkens when exposed to the air; *thallin* gives a greenish-brown color which is marked green in thin layers, and *antipyrin* gives a yellow to blood-red. After the administration of *balsam of copaiva* the urine becomes, when strongly acidified with hydrochloric acid, gradually rose and purple-red (Quincke[4]). After the use of *naphthalin* or *naphthol* the urine gives with concentrated sulphuric acid (1 c. c. concentrated acid and a few drops of urine) a beautiful emerald-green color (Penzoldt[5]), which is probably due to naphthol-glycuronic acid. Odoriferous bodies also pass into the urine. After eating asparagus the urine acquires a sickly disagreeable odor which is probably due to methylmercaptan, according to M. Nencki.[6] After taking turpentine the urine may have a peculiar odor similar to that of violets.

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[1] Arch. f. exp. Path. u. Pharm., Bd. 22.

[2] Zeitschr. f. physiol. Chem., Bd. 18, S. 116.

[3] L. c.

[4] Arch. f. exp. Path. u. Pharm., Bd. 17.

[5] *Ibid.*, Bd. 21.

[6] *Ibid.*, Bd. 28.

VI. Pathological Constituents of Urine.

Proteid. The appearance of slight traces of proteid in the urine of apparently healthy persons has been observed in many cases by several investigators, but still we must not conceal the fact that other investigators consider these traces of proteid as the first symptoms, though very mild, of a diseased condition of the urinary apparatus, or as a symptom of a transitory disturbance in the circulation. Frequently traces are found in the urine of a substance similar to nucleoalbumin which can easily be mistaken for mucin and which is probably identical with nucleoalbumin. This substance has been isolated from the papillary part of the kidneys and from the mucous membrane of the bladder by LÖNNBERG.[1] In diseased conditions proteid occurs in the urine in a variety of cases. The albuminous bodies which most often occur are serglobulin and seralbumin. Albumoses and peptones also sometimes occur. The quantity of proteid in the urine is in most cases less than 5 p. m., rarely 10 p. m., and only very rarely does it amount to 50 p. m. or over.

Among the many reactions proposed for the detection of proteid in urine, the following are to be recommended:

The Heat Test. Filter the urine and test its reaction. An acid urine may, as a rule, be boiled without further treatment, and only in especially acid urines is it necessary to first treat with a little alkali. An alkaline urine is made neutral or faintly acid before heating. If the urine is poor in salts, add $\frac{1}{10}$ vol. of a saturated common-salt solution before boiling; then heat to boiling-point, and if no precipitation, cloudiness, or opalescence appears, the urine in question contains no coagulable proteid, but it may contain albumoses or peptones. If a precipitate is produced on boiling, this may consist of proteid, or of earthy phosphates, or of both. The simple-acid calcium phosphate decomposes on boiling, and normal phosphate may separate. The proper amount of acid is now added to the urine, so as to prevent any mistake caused by the presence of earthy phosphates, and to give a better and more flocculent precipitate of the proteid. If acetic acid is used for this, then add 1–2–3 drops of a 25% acid to each 10 c. c. of the urine, and boil after the addition of each drop. On using nitric acid, add 1–2 drops of the 25% acid to each c. c. of the boiling-hot urine.

On using acetic acid, when the quantity of proteid is very small, and especially when the urine was originally alkaline, the proteid may sometimes remain in solution on the addition of the above quantity of acetic acid. If, on the contrary, less acid is added, the precipitate of calcium phosphate, which forms in amphoteric or faintly acid urines, is liable not to dissolve completely, and this may cause it to be mistaken for a proteid precipitate. If nitric acid is used for the heat test, the fact must not be overlooked that after the addition of only a little acid a combination between it and the proteid is formed which is soluble on boiling and which is only precipitated by an excess of the acid. On this account the large quantity of nitric acid, as suggested above, must be added, but in this case a small part of the proteid is liable to be dissolved by the excess of the nitric acid. When the acid is added after boiling, which is absolutely necessary, the liability of a mistake is not so great. It is on these grounds that the heat test, although it gives very good results in the hands of experts, is not recommended to physicians as a positive test for proteid.

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[1] See page 446.

A confounding with mucin, when this body occurs in the urine, is easily prevented in the heat test with acetic acid, by acidifying another portion with acetic acid at the ordinary temperature. Mucin and nucleoalbumin substances similar to mucin are hereby precipitated. If in the performance of the heat and nitric acid test a precipitate first appears on cooling or is strikingly increased, then this shows the presence of albumoses in the urine, either alone or mixed with coagulable proteid. In this case a further investigation is necessary (see below). In a urine rich in urates a precipitate consisting of uric acid separates on cooling. This precipitate is colored, sandy, and hardly to be mistaken for an albumose or proteid precipitate.

HELLER'S *test* is performed as follows (see page 26): The urine is very carefully floated on the surface of nitric acid in a test-tube. The presence of proteid is shown by a white ring between the two liquids. With this test a red or reddish-violet transparent ring is always obtained with normal urine; it depends on the indigo coloring matters and can hardly be mistaken for the white or whitish proteid ring, and this last must not be mistaken for the ring produced by bile-pigments. In a urine rich in urates another complication may occur, due to the formation of a ring produced by the precipitated uric acid. The uric-acid ring does not lie, like the proteid ring, between the two liquids, but somewhat higher. For this reason we may often have two simultaneous rings with urines rich in urates and yet not containing very much proteid. The disturbance caused by uric acid is easily prevented by diluting the urine with 1–2 vol. water before performing the test. The uric acid now remains in solution, and the delicacy of HELLER'S test is so great that after dilution only in the presence of insignificant traces of proteid does this test give negative results. In a urine very rich in urea a ring-like separation of urea nitrate may also appear. This ring consists of shining crystals, and it does not appear in the previously diluted urine. A confusion with resinous acids, which also give a whitish ring with this test, is easily prevented, since these acids are soluble on the addition of ether. Stir, add ether and carefully shake the contents of the test-tube. If the cloudiness was due to resinous acids, the urine becomes gradually clear and on evaporating the ether a sticky residue of resinous acids is obtained. A liquid which contains pure mucin does not give a precipitate with this test, but it gives a more or less strongly opalescent ring, which disappears on stirring. The liquid does not contain any precipitate after stirring, but is somewhat opalescent. If a faint, not wholly typical reaction is obtained with HELLER'S test after some time with undiluted urine, while the diluted urine gives a pronounced reaction immediately, then, as claimed by K. MÖRNER,[1] a nucleoalbumin substance is present, which is prevented from precipitation by the salts of the undiluted urine. In this case proceed as described below in regard to the detection of nucleoalbumin. If we bear in mind the above-mentioned possible errors and the means by which they may be prevented, there is hardly another test for proteid in the urine which is at the same time so easily performed, so delicate, and so positive as HELLER'S. With this test even 0.02 p. m. albumin may be detected without difficulty. Still the student should not be satisfied with this test alone, but apply at least a second test, such as the heat test. In performing this test the (primary) albumoses are also precipitated.

The *reaction with metaphosphoric acid* (see page 26) is very convenient and easily performed. It is not quite so delicate and positive as HELLER'S test. The albumoses are also precipitated by this reagent.

Reaction with Acetic Acid and Potassium Ferrocyanide. Treat the urine first with acetic acid until about 2%, and then add drop by drop a potassium ferrocyanide solution (1:20), carefully avoiding an excess. This test is very good, and in the hands of experts it is even more delicate than HELLER'S. In the presence of very small quantities of proteid it requires more practice and dexterity than HELLER'S, as the relative quantities of reagent, proteid, and acetic acid influence the result of the test. The quantity of salts in the urine also seems to have an influence. This reagent also precipitates albumoses.

SPIEGLER'S[2] *test.* Spiegler recommends a solution of 8 parts mercuric chloride, 4 parts tartaric acid, 20 parts glycerin, and 200 parts water as a very delicate reagent for proteid in the urine. A test-tube is half filled with this reagent, and the urine allowed to flow upon its surface drop by drop from a pipette along the wall of the test-tube. In the presence of proteid a white ring is obtained at the point of contact between the two liquids. The delicacy of this test is 1:350000.

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[1] Hygiea, Bd. 53. See Maly's Jahresber., Bd. 22, S. 241.

[2] Wien. klin. Wochenschr., 1892, No. 2, and Centralbl. f. klin. Med., 1893, No. 3.

The use of precipitating reagents presumes that the urine to be investigated is perfectly clear, especially in the presence of only very little proteid. The urine must first be filtered. This is not easily done with urine containing bacteria, but a clear urine may be obtained, as suggested by A. Jolles,[1] by shaking the urine with infusorial earth.

The different *color reactions* cannot be directly used, especially in deep-colored urines which only contain little proteid. The common salt of the urine has a disturbing action on Millon's reagent. To prove more positively the presence of proteid, the precipitate obtained in the boiling test may be filtered, washed, and then tested with Millon's reagent. The precipitate may also be dissolved in dilute alkali and the biuret test applied to the solution. The presence of albumoses or peptones in the urine is directly tested for by this last-mentioned test. In testing the urine for proteid one must never be satisfied with one test alone, but one must at least apply the heat test and Heller's test or the potassium-ferrocyanide test. In using the heat test alone the albumoses may be easily overlooked, but these are detected, on the contrary, by Heller's test. If we are satisfied with this last test or the potassium-ferrocyanide test alone, we have no sufficient intimation of the kind of proteid present, whether it consists of albumoses or coagulable proteid.

For practical purposes several dry reagents for proteid have been recommended. Besides the metaphosphoric acid may be mentioned: Stutz's or Fürbringer's gelatin capsules,[2] which contain mercuric choride, sodium chloride, and citric acid; and Geissler's albumin-test papers, which consist of strips of filter-paper which have been dipped in a solution of citric acid and also mercuric-chloride and potassium-iodide solution and then dried.

If the presence of proteid has been positively proved in the urine by the above tests, it then remains necessary to determine the variety.

The detection of *globulin* and *albumin*. In detecting serglobulin the urine is exactly neutralized, filtered, and treated with magnesium sulphate in substance until it is completely saturated at the ordinary temperature, or with an equal volume of a saturated neutral solution of ammonium sulphate. In both cases a white, flocculent precipitate is formed in the presence of globulin. In using ammonium sulphate with a urine rich in urates a precipitate consisting of ammonium urate may separate. This precipitate does not appear immediately, but only after a certain time, and it must not be mistaken for the globulin precipitate. In detecting seralbumin heat the filtrate from the globulin precipitate to boiling-point or add about 1% acetic acid to it at the ordinary temperature.

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[1] Zeitschr. f. anal. Chem., Bd. 29.

[2] In regard to this and other reagents see Huppert-Neubauer's Harnanalyse. 10. Aufl., S. 439.

Albumoses and *peptones* have been repeatedly found in the urine in different diseases. Unquestionable observations are at hand on the occurrence of albumoses in the urine. The statements in regard to the occurrence of peptones[1] date in part from a time when the conception of albumoses and peptones was different from that of the present day and in part they are based upon investigations using insufficient methods. It is difficult to give anything positive in regard to the occurrence of so-called true peptone in the urine, and the study of peptonuria seems to require thorough investigation.

In detecting *albumoses* first remove all coagulable proteids by boiling with the addition of acetic acid. The filtrate is then tested by the biuret test, and when this gives positive results apply the three previously mentioned albumose reagents (page 34), nitric acid, acetic acid and potassium ferrocyanide, and saturation with common salt with the addition of acid. The albumoses may also be precipitated by saturating with ammonium sulphate in substance, and the detection of albumoses as well as true peptones is best performed by the aid of this salt. According to DEVOTO[2] we proceed as follows:

DEVOTO'S *method*. The coagulable proteid is precipitated by ammonium sulphate as directed on page 29. The precipitate also contains the albumoses. If true peptone is present, it is found in the filtrate and may be tested for therein by means of the biuret test. The precipitate is washed with a saturated solution of ammonium sulphate and then treated with water. The coagulable proteid remains undissolved, while the albumoses dissolve and may be tested for by the biuret test. The deutero-albumose are nevertheless not completely precipitated by the ammonium sulphate, and a mistaking of this for true peptone may occur.

In testing for peptone in the old sense we make use of SALKOWSKI'S[3] modification of HOFMEISTER'S[4] method. 50 c. c. of the urine to be tested is acidified with 5 c. c. hydrochloric acid, precipitated with phospho-tungstic acid and warmed on a wire gauze. As soon as the precipitate is converted to a resinous mass the liquid is poured off as well as possible and the mass washed twice with distilled water. It is then dissolved in about 8 c. c. water by the aid of 0.5 c. c. caustic soda of sp. gr. 1.16 and warmed until the blue solution is decolorized (grayish yellow or yellow). This solution is used after cooling for the biuret test by the addition of a copper solution (1-2%) drop by drop.

At the present time we have no trustworthy method for the quantitative estimation of albumoses and peptones in the urine.

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[1] In regard to the literature on albumoses and peptones in urine see Huppert-Neubauer-Harnanalyse, 10. Aufl., S. 466 to 492; also A. Stoffregen, Ueber das Vorkommen von Pepton im Harn, Sputum und Eiter. Inaug.-Diss. Dorpat, 1891; H. Hirschfeldt, Ein Beitrag zur Frage der Peptonurie. Inaug.-Diss. Dorpat, 1892; and especially Stadelmann, Untersuchungen über die Peptonurie. Wiesbaden, 1894.

[2] Zeitschr. f. physiol. Chem., Bd. 15.

[3] Centralbl. f. d. med. Wissensch., 1894.

[4] Zeitschr. f. physiol. Chem., Bd. 4.

Quantitative Estimation of Proteid in Urine. Of all the methods proposed thus far, the COAGULATION METHOD (boiling with the addition of acetic acid) when performed with sufficient care gives the best results. The average errors need never amount to more than 0.01%, and it is generally smaller. In using this method it is best to first find how much acetic acid must be added to a small portion of urine, which has been previously heated on the water-bath, to completely separate the proteid, so that the filtrate does not respond to HELLER'S test. Then coagulate 20–50–100 c. c. of the urine. Pour the urine into a beaker and heat on the water-bath, add the required quantity of acetic acid slowly, stirring constantly, and heat at the same time. Filter while warm, wash first with water, then with alcohol and ether, dry and weigh, ash and weigh again. In exact determinations the filtrate must not give HELLER'S test.

The above-mentioned method of DEVOTO may also be used in the quantitative estimation of coagulable proteids. The error originating from the precipitation of uric acid and other urinary constituents by the ammonium sulphate is so very small in ordinary cases where the precipitate is carefully washed that it is unimportant (REDELIUS[1]). In the presence of only little proteid in a urine rich in uric acid it may on the contrary be quite considerable.

The separate estimation of GLOBULINS and ALBUMINS is done by carefully neutralizing the urine and prepcipitating with $MgSO_4$ added to saturation (AUTHOR), or simply by adding an equal volume of a saturated neutral solution of ammonium sulphate (HOFMEISTER and POHL[2]). The precipitate consisting of globulin is thoroughly washed with a saturated magnesium sulphate or half-saturated ammonium-sulphate solution, dried continuously at 110° C., boiled with water, extracted with alcohol and ether, then dried, weighed, ashed, and weighed again. The quantity of albumin is calculated as the difference between the quantity of globulins and the total proteids.

Approximate Estimation of Proteid in Urine. Of the methods suggested for this purpose none has been more extensively employed than ESBACH'S.

ESBACH'S[3] *method.* The acidified urine (acidified with acetic acid) is poured into a specially graduated tube to a certain mark and then the reagent (a 2% citric-acid and 1% picric-acid solution in water) is added to a second mark, the tube is closed with a rubber stopper and carefully shaken, avoiding the production of froth. The tube is allowed to stand twenty-four hours, and then the height of the precipitate in the graduated tube is read off. The reading gives directly the quantity of proteid in 1000 parts of the urine. Urines rich in proteid must first be diluted with water. The results obtained by this method are, however, dependent upon the temperature; and a difference in temperature of 5° to 6.5° C. may in urines containing a medium quantity of proteid cause an error of 0.2–0.3% deficiency or excess (CHRISTENSEN and MYGGE). This method is only to be used in a room in which the temperature may be kept nearly constant. The directions for the use of the apparatus accompany it.

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[1] Upsala Lakarefs Förh., Bd. 27, and Maly's Jahresber., Bd. 22.

[2] Arch. f. exp. Path. u. Pharm., Bd. 20.

[3] In regard to the literature on this method and the numerous experiments to determine its value see Huppert-Neubauer, 10. Aufl., S. 853.

CHRISTENSEN'S and MYGGE'S[1] *method.* 5 c. c. of urine, after being acidified with 2 drops of acetic acid, are poured into a somewhat modified burette and precipitated with a certain quantity of a 1% tannic-acid solution and then treated with 1 c. c. of mucilage. After the addition of water to a certain mark and after inverting the tube several times a uniform emulsion is produced. A cylindrical glass filled one half or one third with water is now placed on a white surface having a number of close black lines traced upon it, and the contents of the burette are gradually added to the water with constant stirring, until by close observation the black lines cannot even be distinguished from the white spaces. The reading of the quantity of urine emulsion employed gives directly the quantity of proteid in the urine. This method is claimed to give very good results. A special description accompanies each apparatus.[2]

The method proposed by ROBERTS and STOLNIKOW and further developed by BRANDBERG, though somewhat more difficult to perform, also gives satisfactory results. The density methods of LANG, HUPPERT, and ZAHOR[3] are also very good. The last consists in determining the specific gravity before and after the coagulation of the proteids.

Nucleoalbumin and **Mucin.** Nucleoalbumin seems to be a regular constituent of urine, although ordinarily it only occurs in very small quantities. Mucin is alleged to occur in small quantities under normal conditions, but appears in greater quantities in catarrhal affections of the urinary passages. There is no doubt[4] that cases exist in which true mucin occurs in the urine; in most cases, nevertheless, we are doubtless dealing with a nucleoalbumin similar to mucin, which originates in the kidneys or urinary passages.[5]

To detect mucin in urine, it must first be diluted with water to prevent a precipitation of the uric acid on subsequent addition of acid, and also to reduce the solvent action of the common salt of the urine on the mucin. Now add an excess of acetic acid. The precipitate formed is purified by dissolving in water with the addition of a little alkali and reprecipitated with acetic acid. The precipitate is tested with the ordinary mucin reagents. To avoid mistaking mucin for nucleoalbumin, which is similar to mucin, the precipitate must be tested in regard to its behavior on boiling with dilute mineral acids. If no reducing substance is formed by this treatment, it contains no mucin. To detect nucleoalbumin we proceed in the same manner, but it is better to remove the salts from the urine by means of dialysis (K. MÖRNER[1]). Then precipitate with not too much acetic acid. To determine if the precipitate consists of nucleoalbumin or a nucleoproteid, we test for xanthin bases after boiling with an acid. Large quantities of the precipitate are necessary for this purpose.

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[1] See Maly's Jahresber., Bd. 18, S. 314.

[2] The apparatus may be obtained from C. Knudsen in Copenhagen.

[3] In regard to these methods see Huppert-Neubauer's Harnanalyse, 10. Aufl., S. 845–853.

[4] See B. Malfatti, Maly's Jahresber., Bd. 21, S. 22.

[5] In regard to the literature see Huppert-Neubauer, S. 540; Lönnberg, Upsala Läkarefs Förh., Bd. 25; K. Mörner, Hygiea, Bd. 53; Obermayer, Centralbl. f. klin. Med., Bd. 13.

Blood and Blood-coloring Matters. The urine may contain blood from hemorrhage in the kidneys or other parts of the urinary passages (HÆMATURIA). In these cases, when the quantity of blood is not very small, the urine is more or less cloudy and colored reddish, yellowish red, dirty red, brownish red, or dark brown. In recent hemorrhages, in which the blood has not decomposed, the color is nearer blood-red. Blood-corpuscles may be found in the sediment, sometimes also blood-casts and smaller or larger blood-clots.

In certain cases the urine contains no blood-corpuscles, but only dissolved blood-coloring matters, hæmoglobin or, and indeed quite often, methæmoglobin (HÆMOGLOBINURIA). The blood-pigments appear in the urine under different conditions, as in dissolution of blood in poisoning with arseniuretted hydrogen, chlorates, etc., after serious burns, after transfusion of blood, and also in the periodic appearance of hæmoglobinuria with fever. The urine may in hæmoglobinuria also have an abundant grayish-brown sediment rich in proteid which contains the remains of the stromata of the red blood-corpuscles. In animals hæmoglobinuria may be produced by many causes which force free hæmoglobin into the plasma.

To detect blood in the urine we make use of the microscope, spectroscope, the guaiacum test, and HELLER'S or HELLER-TEICHMANN'S test.

Microscopic Investigation. The blood-corpuscles may remain undissolved for a long time in acid urine; in alkaline urine, on the contrary, they are easily changed and dissolved. They often appear entirely unchanged in the sediment; in some cases they are distended, and in others unequally pointed or jagged like a thorn-apple. In hemorrhage of the kidneys a cylindrical clot is sometimes found in the sediment, which is covered with numerous red blood-corpuscles, forming casts of the urinary passages. These formations are called BLOOD-CASTS.

The *spectroscopic investigation* is naturally of very great value; and if it be necessary to determine not only the presence but also the kind of coloring matter, this method is indispensable. In regard to the optical behavior of the various blood-pigments we must refer to Chapter VI.

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[1] K. Mörner, Hygiea, Bd. 53.

Guaiacum Test. Mix in a test-tube equal volumes of tincture of guaiacum and old turpentine which has become strongly ozonized by the action of air under the influence of light. To this mixture, which must not have the slightest blue color, add the urine to be tested. In the presence of blood or blood-pigments, first a bluish-green and then a beautiful blue ring appears where the two liquids meet. On shaking the mixture it becomes more or less blue. Normal urine or one containing proteid does not give this reaction. For the explanation of this we must refer the reader to Chapter VI, page 134. Urine containing pus, although no blood is present, gives a blue color with these reagents; but in this case the tincture of guaiacum alone, without turpentine, is colored blue by the urine (VITALI[1]). This is at least true for a tincture that has been exposed for some time to the action of air and sunlight. The blue color produced by pus differs from that produced by blood-coloring matters by disappearing on heating the urine to boiling. A urine alkaline by decomposition must first be made faintly acid before performing the reaction. The turpentine should be kept exposed to sunlight, while the tincture of guaiacum must be kept in a dark glass bottle. These reagents to be of use must be controlled by a liquid containing blood. This test, it is true, in positive results is not absolutely decisive, because other bodies may give a blue reaction; but when properly performed it is so extremely delicate that when it gives negative results any other test for blood is superfluous.

HELLER-TEICHMANN'S *Test.* If a neutral or faintly acid urine containing blood is heated to boiling, we always obtain a mottled precipitate consisting of albumin and hæmatin. If caustic soda is added to the boiling-hot test, the liquid becomes clear and turns green when examined in thin layers (due to hæmatin alkali), and a red precipitate, appearing green by reflected light, re-forms which consists of earthy phosphates and hæmatin. This reaction is called HELLER'S blood-test. If this precipitate is collected after a time on a small filter, it may be used for the hæmin test (see page 143). If the precipitate contains only a little blood-coloring matter with a larger quantity of earthy phosphates, then wash it with dilute acetic acid, which dissolves the earthy phosphates, and use the residue for the preparation of TEICHMANN'S hæmin crystals. If, on the contrary, the amount of phosphates is very small, then first add a little $CaCl_2$ solution to the urine, heat to boiling, and add simultaneously with the caustic potash some sodium-phosphate solution. In the presence of only very small quantities of blood, first make the urine very faintly alkaline with ammonia, add tannic acid, acidify with acetic acid, and use the precipitate in the preparation of the hæmin crystals (STRUVE[1]).

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[1] See Maly's Jahresber., Bd. 18, S. 326.

Hæmatoporphyrin. Since the occurrence of hæmatoporphyrin in the urine in various diseases has been made very probable by several investigators, such as NEUSSER, STOKVIS, MACMUNN, LE NOBEL, RUSSEL, COPEMAN, and others,[2] SALKOWSKI[3] has positively shown the presence of this pigment in the urine after sulphonal intoxications. It was first isolated in a pure crystalline state by the AUTHOR[4] from the urine of insane women after sulphonal intoxication. According to GARROD[5] traces of hæmatoporphyrin occur regularly in normal urines. It is also found in the urine during different diseases, although it only occurs in small quantities. It has been found in considerable quantities in the urine after intoxication with sulphonal.

Urine containing hæmatoporphyrin is sometimes only slightly colored, while in other cases, as for example after the use of sulphonal, it is more or less deep red in color. The color depends in these last-mentioned cases, in greatest part, not upon hæmatorpophyrin, but upon other red or reddish-brown pigments, which have not been sufficiently studied. The pathogenic moment of hæmatoporphyrinuria is according to STOKVIS[6] an absorption and elimination of the blood emptied into the intestinal tract or present there and changed into hæmatoporphyrin.

In detecting hæmatoporphyrin the urine is precipitated with alkaline barium-chloride solution (a mixture of equal volumes of a barium-hydrate solution, saturated in the cold, and a 10% barium-chloride solution according to SALKOWSKI), or the urine is made strongly alkaline with a soda solution, according to GARROD, which precipitates the earthy phosphates. In both cases the hæmatoporphyrin is carried down with the precipitate, while urobilin and certain other pigments remain in solution. The washed precipitate is allowed to stand some time at the temperature of the room with alcohol containing hydrochloric or sulphuric acid and then filtered. The filtrate shows the characteristic spectrum of hæmatoporphyrin in acid solution, and gives the spectrum of alkaline hæmatoporphyrin after saturation with ammonia. If the alcoholic solution is mixed with chloroform and a large quantity of water added and carefully shaken, sometimes a lower layer of chloroform is obtained which contains very pure hæmatoporphyrin, while the upper layer of alcohol and water contains the other pigments besides some hæmatoporphyrin.

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[1] Zeitschr. f. anal. Chem., Bd. 11.

[2] A very complete index of the literature on hæmatoporphyrin in the urine may be found by R. Zoja, Su qualche pigmento di alcune urine, etc., in Arch. Ital. di clin. Med., 1893.

[3] Zeitschr. f. Physiol. Chem., Bd. 15.

[4] Skand. Arch. f. Physiol., Bd. 3.

[5] Journal of Physiol., vols. 13 and 17.

[6] Zeitschr. f. klin. Med., Bd. 28.

BAUMSTARK[1] found in a case of leprosy two characteristic coloring matters in the urine, "urorubrohæmatin" and "urofuscohæmatin," which, as their names indicate, seem to stand in close relationship to the blood-coloring matters. *Urorubrohæmatin*, $C_{68}H_{94}N_8Fe_2O_{26}$, contains iron and shows an absorption-band in front of *D* and a broader one back of *D*. In alkaline solution it shows four bands, behind *D*, at *E*, beyond *F*, and behind *G*. It is not soluble either in water, alcohol, ether, or chloroform. It gives a beautiful brownish-red non-dichroitic liquid with alkalies. *Urofuscohæmatin*, $C_{68}H_{106}N_8O_{26}$, which is free from iron, shows no characteristic spectrum; it dissolves in alkalies, producing a brown color. It remains to be proved whether these two pigments are related to (impure) hæmatoporphyrin.

Melanin. In the presence of melanotic cancers dark coloring matters are sometimes eliminated with the urine. K. MÖRNER[2] has isolated two pigments from such a urine, of which one was soluble in warm 50–75% acetic acid and the other, on the contrary, was insoluble. The one seemed to be *phymatorhusin* (see Chapter XVI). Usually the urine does not contain any melanin, but a chromogen of melanin, a *melanogen*. In such cases the urine gives EISELT'S reaction, becoming dark-colored with oxidizing agents such as conc. nitric acid, potassium bichromate, and sulphuric acid, as well as with free sulphuric acid. Urine containing melanin or melanogen is colored black by ferric-chloride solution (v. JAKSCH[3]).

Urorosein, so named by NENCKI,[4] is a urinary coloring matter, occurring in various diseases, which appears on the acidification of the urine with a mineral acid, and which is taken up by shaking with amyl-alcohol. The solution shows an absorption-band between *D* and *E*. This pigment, which is not soluble in chloroform or ether, is not identical with indigo-red. Alkalies decolorize the solution of this pigment immediately, and it is also rather quickly bleached by light. According to ZAWADSKI[5] urorosein is derived from urobilin by oxidation. *Uroerythrin*, which gives a rose-red color to the urinary sediments especially in fevers, seems to occur also in urine under physiological conditions.

Pus occurs in the urine in different inflammatory affections, especially in catarrh of the bladder and in inflammation of the membrane of the kidneys or the urethra.

Pus is best detected by means of the microscope. The pus-cells are rather easily destroyed in alkaline urines. In detecting pus we make use of DONNÉ'S pus-test, which is performed in the following way: Pour off the urine from the sediment as carefully as possible, place a small piece of caustic alkali on the sediment, and stir. If the pus-cells have not been previously changed, the sediment is converted by this means into a slimy tough mass.

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[1] Pflüger's Arch., Bd. 9.

[2] Zeitschr. f. physiol. Chem., Bd. 11.

[3] *Ibid.*, Bd. 13.

[4] Nencki und Sieber, Journal f. prakt. Chem. (N. F.), Bd. 26.

[5] Arch. f. exp. Path. u. Pharm., Bd. 28.

The pus-corpuscles swell up in alkaline urines, dissolve, or at least are so changed that they cannot be recognized under the microscope. The urine in these cases is more or less slimy or fibrous, and it is precipitated in large flakes by acetic acid, so that it may possibly be mistaken for mucin. The closer investigation of the precipitate produced by acetic acid, and especially the appearance or non-apppearance of a reducing substance after boiling it with a mineral acid, demonstrates the nature of the precipitated substance. Urine containing pus always contains proteid.

Bile-acids. The statements in regard to the occurrence of bile-acids in the urine under physiological conditions do not agree. According to DRAGENDORFF and HÖNE traces of bile-acids occur in the urine; according to MACKAY and v. UDRÁNSZKY,[1] they do not. Pathologically they are present in the urine in hepatogenic icterus, although not always.

Detection of Bile-acids in the urine. PETTENKOFER'S test gives the most decisive reaction; but as it gives similar color reactions with other bodies, it must be supplemented by the spectroscopic investigation. The direct test for bile-acids is easy after the addition of traces of bile to a normal urine. But the direct detection in a colored icteric urine is more difficult and gives very misleading results; the bile-acid must therefore always be isolated from the urine. This may be done by the following method of HOPPE-SEYLER, which is slightly modified in non-essential points.

HOPPE-SEYLER'S METHOD. Strongly concentrate the urine, and extract the residue with strong alcohol. The filtrate is freed from alcohol by evaporation and then precipitated by basic lead acetate and ammonia. The washed precipitate is treated with boiling alcohol, filtered hot, the filtrate treated with a few drops of soda solution, and evaporated to dryness. The dry residue is extracted with absolute alcohol, filtered, and an excess of ether added. The amorphous or, after a longer time, crystalline precipitate consisting of alkali-salts of the biliary acids is used in performing PETTENKOFER'S test.

Bile-coloring matters occur in the urine in different forms of icterus. A urine containing bile-coloring matters is always abnormally colored—yellow, yellowish brown, deep brown, greenish yellow, greenish brown, or nearly pure green. On shaking it froths, and the bubbles are yellow or yellowish green in color. As a rule icteric urine is somewhat cloudy, and the sediment is frequently, especially when it contains epithelium-cells, rather strongly colored by the bile-pigments. In regard to the occurrence of urobilin in icteric urine see page 501.

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[1] Cited from Huppert-Neubauer, Harnanalyse, 10. Aufl., S. 229.

Detection of bile-coloring matters in urine. Many tests have been proposed for the detection of bile-coloring matters. Ordinarily we obtain the best results either with GMELIN's or with HUPPERT's test.

GMELIN's *test* may be applied directly to the urine; but it is better to use ROSENBACH's modification. Filter the urine through a very small filter, which is deep-colored from the retained epithelium-cells and bodies of that kind. After the liquid has entirely passed through apply to the inside of the filter a drop of nitric acid which contains only very little nitrous acid. A pale-yellow spot will be formed which is surrounded by colored rings which appear yellowish red, violet, blue, and green from within outward. This modification is very delicate, and it is hardly possible to mistake indican and other coloring matters for the bile-pigments. Several other modifications of GMELIN's test on the urine directly, as with concentrated sulphuric acid and nitrate, etc., have been proposed, but they are neither simpler nor more delicate than ROSENBACH's modification.

HUPPERT's *Reaction*. In a dark-colored urine or one rich in indican we do not always obtain good results with GMELIN's test. In such cases, as also in urines containing blood-coloring matters at the same time, the urine is treated with lime-water, or first with some $CaCl_2$ solution, and then with a solution of soda or ammonium carbonate. The precipitate which contains the bile-coloring matters is filtered and used for HUPPERT's test (see page 235).

The precipitate consisting of lime-pigments may also be shaken out with chloroform after washing in water and after being acidified with acetic acid. The bilirubin is taken up by the chloroform, which is colored yellow thereby, while the acetic-acid solution is colored green by the biliverdin. Both solutions may then be used for GMELIN's test (HOPPE-SEYLER), and small quantities of bile-coloring matters may be detected in this way. The lime-pigments may, according to HILGER, also be used directly for GMELIN's test in the following way: Spread them on a porcelain dish in a thin layer, and add carefully a drop of nitric acid. The reaction generally appears very beautiful.

JOLLES' *Method*.[1] Place 50 c. c. of the urine in a cylinder with a glass stopper, add a few drops of 10% hydrochloric acid and an excess of a barium-chloride solution with 5 c. c. chloroform, and shake thoroughly for a few minutes. After about 10 minutes remove the chloroform and the precipitate by means of a pipette and place in a test-tube and heat on the water-bath at about 80° C.

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[1] Zeitschr. f. physiol. Chem., Bd 18, S. 545. This contains the literature on all the known tests for bile-pigments with the exception of Stokvis's test, which may be found in Maly's Jahresber., Bd. 12, S. 226.

the pus-cells have not been previously changed, the sediment is converted by this means into a slimy tough mass.

The pus-corpuscles swell up in alkaline urines, dissolve, or at least are so changed that they cannot be recognized under the microscope. The urine in these cases is more or less slimy or fibrous, and it is precipitated in large flakes by acetic acid, so that it may possibly be mistaken for mucin. The closer investigation of the precipitate produced by acetic acid, and especially the appearance or non-apppearance of a reducing substance after boiling it with a mineral acid, demonstrates the nature of the precipitated substance. Urine containing pus always contains proteid.

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HOPPE-SEYLER'S METHOD. Strongly concentrate the urine, and extract the residue with strong alcohol. The filtrate is freed from alcohol by evaporation and then precipitated by basic lead acetate and ammonia. The washed precipitate is treated with boiling alcohol, filtered hot, the filtrate treated with a few drops of soda solution, and evaporated to dryness. The dry residue is extracted with absolute alcohol, filtered, and an excess of ether added. The amorphous or, after a longer time, crystalline precipitate consisting of alkali-salts of the biliary acids is used in performing PETTENKOFER'S test.

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[1] Cited from Huppert-Neuba

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GMELIN's *test* may be applied directly to the urine; but it is better to use ROSENBACH's modification. Filter the urine through a very small filter, which is deep-colored from the retained epithelium-cells and bodies of that kind. After the liquid has entirely passed through apply to the inside of the filter a drop of nitric acid which contains only very little nitrous acid. A pale-yellow spot will be formed which is surrounded by colored rings which appear yellowish red, violet, blue, and green from within outward. This modification is very delicate, and it is hardly possible to mistake indican and other coloring matters for the bile-pigments. Several other modifications of GMELIN's test on the urine directly, as with concentrated sulphuric acid and nitrate, etc., have been proposed, but they are neither simpler nor more delicate than ROSENBACH's modification.

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After the evaporation of the chloroform carefully decant the liquid from the precipitate and allow 3 drops concentrated nitric acid containing $\frac{1}{3}$ fuming nitric acid to flow down the sides of the test-tube. In the presence of bile-pigments the characteristic colored rings are obtained, and this modification, according to JOLLES, is the most delicate of all tests for bile-pigments.

STOKVIS's *reaction* is especially valuable in those cases in which the urine contains only very little bile-coloring matter together with larger quantities of other coloring matters. The test is performed as follows: 20–30 c. c. urine are treated with 5–10 c. c. of a solution of zinc acetate (1 : 5). The precipitate is washed on a small filter with water and then dissolved in a little ammonia. The new filtrate gives, directly or after it has stood a short time in the air until it has a peculiar brownish-green color, the absorption-bands of bilicyanin (see page 235).

Many other reactions for bile-coloring matters in the urine have been proposed; but as the above-mentioned are sufficient, it is perhaps only necessary to give here a few of the other reactions, without entering into details.

ULTZMANN's *reaction* consists in treating about 10 c. c. of the urine with 3–4 c. c. concentrated caustic-potash solution and then acidifying with hydrochloric acid. The urine will become a beautiful green.

SMITH's *Reaction*. Pour carefully over the urine tincture of iodine, whereby a green ring appears between the two liquids. You may also shake the urine with tincture of iodine until it has a green color.

EHRLICH's *Test*. First mix the urine with an equal volume of dilute acetic acid and then add drop by drop a solution of sulpho-diazobenzol. The acid mixture becomes dark red in the presence of bilirubin, and this color becomes bluish violet on the addition of glacial acetic acid. The sulpho-diazobenzol is prepared with 1 grm. sulphanilic acid, 15 c. c. hydrochloric acid, and 0.1 grm. sodium nitrite; this solution is diluted to 1 litre with water.

MEDICINAL COLORING MATTERS produced from santonin, rhubarb, senna, etc., may give an abnormal color to the urine which may be mistaken for bile-coloring matters or, in alkaline urines, perhaps for blood-coloring matters. If hydrochloric acid is added to such a urine, it becomes yellow or pale yellow, while on the addition of an excess of alkali it becomes more or less beautifully red.

Sugar in Urine.

The occurrence of traces of grape-sugar in the urine of perfectly healthy persons has been, as above stated (page 505), quite positively proved. If sugar appears in the urine in constant and especially in large quantities, it must be considered as an abnormal constituent. We have given in a previous chapter several of the most important conditions which cause glycosuria in man and animals, and we must refer the reader to Chapters VIII and IX for the essential facts in regard to the appearance of sugar in the urine.

In man the appearance of glucose in the urine has been observed in numerous and various pathological conditions, such as lesions of the brain and especially of the medulla oblongata, abnormal circulation in the abdomen, diseases of the heart and lungs, diseases of the liver, cholera, and many other diseases. The continued presence of sugar in human urine, sometimes in very considerable quantities, occurs in DIABETES MELLITUS. In this disease there may be an elimination of 1 kilogramme or even more of grape-sugar during the 24 hours. In the beginning of the disease, when the quantity of sugar is still very small, the urine often does not appear abnormal. In more developed, typical cases the quantity of urine voided increases considerably, to 3–6–10 litres per 24 hours. The percentage of the physiological constituents is as a rule very low, while their absolute daily quantity is increased. The urine is pale, but of a high specific gravity, 1.030–1.040 or even higher. The high specific gravity depends upon the quantity of sugar present, which varies in different cases, but may be as high as 10%. The urine is therefore characterized in typical cases of diabetes by the very large quantity voided, by the pale color and high specific gravity, and by its containing sugar.

That the urine after the introduction of certain medicines or poisonous bodies into the system contains reducing bodies, conjugated glycuronic acids, which may be mistaken for sugar, has already been mentioned.

The properties and reactions of glucose have been treated of in a previous chapter, and it remains but to mention the methods of detecting and quantitatively estimating glucose in the urine.

The *detection of sugar* in the urine is ordinarily, in the presence of not too small quantities of sugar, a very simple task. The presence of only very small quantities may make its detection sometimes very difficult and laborious. A urine containing proteid must first have the proteid removed by coagulation with acetic acid and heat before it can be tested for sugar.

The tests which are most frequently employed and are especially recommended are as follows:

TROMMER'S *Test*. In a typical diabetic urine or one rich in sugar this test succeeds well, and it may be performed in the manner suggested on page 69. This test may lead to very great mistakes in urines poor in sugar, especially when they have at the same time normal or increased amounts of physiological constituents, and therefore it cannot be recommended to physicians or to persons inexperienced in such work. Normal urine contains reducing substances, such as uric acid, creatinin, and others, and therefore a reduction takes place with all urine on using this test. We do not generally have a separation of copper suboxide, but still if we vary the proportion of the alkali to the copper sulphate and boil we often have an actual separation of suboxide in normal urines, or we obtain a peculiar yellowish-red liquid due to finely divided hydrated suboxide. This occurs especially on the addition of much alkali or too much copper sulphate, and by careless manipulation the inexperienced worker may therefore sometimes obtain apparently positive results in a normal urine. On the other hand, as urine contains substances, such as creatinin and ammonia (from the urea), which in the presence of only little sugar may keep the copper suboxide in solution, he may easily overlook small quantities of sugar that may be present.

TROMMER's test may of course be made positive and useful, even in the presence of very small quantities of sugar, by using the modification suggested by WORM MÜLLER. As this modification is rather complicated, and requires much practice and exactness, it is probably rarely employed by the busy physician. The following test is to be preferred:

ALMÉN's *bismuth test*, which recently has been incorrectly called NYLANDER's test, is performed with the alkaline bismuth solution prepared as above described (page 69). For each test 10 c. c. of urine are taken and treated with 1 c. c. of the bismuth solution and boiled for a few minutes. In the presence of sugar the urine becomes darker yellow or yellowish brown. Then it grows darker, cloudy, dark brown, or nearly black, and non-transparent. After a shorter or longer time a black deposit appears, the supernatant liquid gradually clears, but still remains colored. In the presence of only very little sugar the test is not black or dark brown, but simply deeper-colored, and not until after some time do we see on the upper layer of the phosphate precipitate a dark or black edge (of bismuth?). In the presence of much sugar a larger amount of reagent may be used without disadvantage. In a urine poor in sugar we must use only 1 c. c. of the reagent for every 10 c. c. of the urine.

This test shows the presence of 1–0.5 p. m. sugar in the urine. The sources of error which interfere in TROMMER's test, such as the presence of uric acid and creatinin, entirely disappear in this test. The bismuth test is, besides, more easily performed, and it is therefore to be recommended to the physician. Small quantities of proteid do not interfere with this test; large quantities may give rise to an error by forming bismuth sulphide, and therefore must be removed by coagulation.

In using this method it must not be overlooked that it is, like TROMMER's test, a reduction test, and it consequently may show, besides sugar, certain other reducing substances. Such bodies are certain conjugated glycuronic acids which may appear in the urine. Positive results have been obtained with the bismuth test on urine after the use of several medicines such as rhubarb, senna, antipyrin, kairin, salol, turpentine, and others. From this it follows that we should never be satisfied with this test alone, especially when the reduction is not very great. When this test gives negative results we can consider the urine as free from sugar from a clinical standpoint, and when it gives positive results other tests must be applied. Among these the fermentation test is of special value.

Fermentation Test. On using this test we must proceed in various ways, according as the bismuth test shows small or large quantities. If a rather strong reduction is obtained, the urine may be treated with yeast and the presence of sugar determined by the generation of carbon dioxide. In this case the acid urine, or that faintly acidified with tartaric acid, is treated with yeast which has previously been washed by decantation with water. Pour this urine to which the yeast has been added into a SCHRÖTTER's gas-burette, or glass tube with the open end ground, close with the thumb, and open under the surface of mercury contained in a dish. As the fermentation proceeds, the carbon dioxide collects in the upper part of the tube, while a corresponding quantity of liquid is expelled below. As a control in this case two other similar tests must be made, one with normal urine and yeast to learn the quantity of gas usually developed, and the other with a sugar solution and yeast to determine the activity of the yeast.

If, on the contrary, we find only a faint reduction with the bismuth test, no positive conclusion can be drawn from the absence of any carbon dioxide or the appearance of a very insignificant quantity. In this case proceed in the following way: Treat the acid urine, or the urine which has been faintly acidified with tartaric acid, with yeast whose activity has been tested by a special test on a sugar solution, and allow it to stand 24–48 hours at the temperature of the room, or, better, at a little higher temperature. After this time test again with the bismuth test, and if the reaction now gives negative results, then sugar was previously present. But if the reaction continues to give positive results, then it shows—if the yeast is active—the presence of other reducing, unfermentable bodies. There remains of course the possibility that the urine also contains some sugar besides these bodies. This possibility may be determined by the following test:

Phenylhydrazin Test. According to v. JAKSCH,[1] this test is performed in the following way: Add in a test-tube containing 8–10 c. c. of the urine two knife-points of phenylhydrazin hydrochloride and three knife-points sodium acetate, and when the added salts do not dissolve on warming add more water. The mixture is heated in boiling water and kept there for one hour to avoid a confusion with phenylhydrazin-glycuronic acid (v. JAKSCH and HIRSCHL). It is then poured into a beaker of cold water. If the quantity of sugar present is not too small, a yellow crystalline precipitate is now obtained. If the precipitate appears amorphous, there are found, on looking at it under the microscope, yellow needles singly and in groups. If very little sugar is present, pour the test into a conical glass and examine the sediment. In this case at least a few phenylglucosazone crystals are found, while the occurrence of smaller and larger yellow plates or highly refractive brown globules do not show the presence of sugar. According to v. JAKSCH, this reaction is very reliable, and by it the presence of 0.3 p. m. sugar can be detected (ROSENBERG,[1] GEYER[2]).

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[1] v. Jaksch, Klin. Diagnostik, 4. Aufl., S. 375.

The value of this test has been considerably debated, and the objection has been made that glycuronic acid also gives a similar precipitate. A confounding with glycuronic acid is, according to HIRSCHL,[3] not to be apprehended when it is not heated in the water-bath for too short a time (one hour). KISTERMANN[4] found this precaution insufficient, and ROOS[5] states that the phenylhydrazin test always gives a positive result with human urine. In doubtful cases where we wish to be quite positive, prepare the crystals from a large quantity of urine, dissolve them on the filter by pouring over them hot alcohol, treat the filtrate with water, and boil off the alcohol. If the characteristic yellow crystalline needles, whose melting-point (204–205° C.) is also determined, are now obtained, then this test is decisive for the presence of sugar. It must not be forgotten that lævulose gives the same osazone as grape-sugar, and that a further investigation is necessary in certain cases.

Polarization. This test differentiates between dextrose, which polarizes to the right, and lævulose, which polarizes to the left. The polariscopic investigation is of great value, especially as in many cases it quickly differentiates between sugar and other reducing, lævogyrate substances, such as conjugated glycuronic acid. In the presence of only very little sugar the value of this test depends on the delicacy of the instrument and the dexterity of the observer; therefore this method is perhaps inferior in most cases to the bismuth test or to the phenylhydrazin test.

If small quantities of sugar are to be isolated from the urine, precipitate the urine first with sugar of lead, filter, precipitate the filtrate with ammoniacal basic lead acetate, wash this precipitate with water, decompose it with H_2S when suspended in water, concentrate the filtrate, treat it with strong alcohol until it is 80 vol. per cent, filter when necessary, and add an alcoholic caustic-alkali solution. Dissolve the precipitate consisting of saccharates in a little water, precipitate the potash by an excess of tartaric acid, neutralize the filtrate with calcium carbonate in the cold, and filter. The filtrate may be used for testing with the polariscope as well as in the fermentation, bismuth, and phenylhydrazin tests. The presence of grape-sugar may be detected by this same process in animal fluids or tissues from which the proteids have been removed by coagulation or by the addition of alcohol.

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[1] Deutsch. med. Wochenschr., 1888.

[2] Wien. med. Presse, 1889, S. 1688. Cited from Roos, Zeitschr. f. physiol. Chem., Bd. 15, S. 524.

[3] Zeitschr. f. physiol. Chem., Bd. 14.

[4] Deutsch. Arch. f. klin. Med., Bd. 50. Cited from Maly's Jahresber., Bd. 22, S. 229.

[5] Zeitschr. f. physiol. Chem., Bd. 15.

For the physician, who naturally wants specially simple and quick methods, the bismuth test must be especially recommended. If this test gives negative results, the urine is to be considered as free from sugar in a clinical sense. If it gives positive results, the presence of sugar must be controlled by other tests, especially by the fermentation test.

Other tests for sugar, as, for example, the reaction with orthonitrophenylpropiolic acid, picric acid, diazobenzol-sulphonic acid, are superfluous. The reaction with α-naphthol, which is a reaction for carbohydrates in general, for glycuronic acid and mucin, may, because of its extreme delicacy, give rise to mistakes, and is therefore not to be recommended to physicians. Normal urines give this test, and if the strongly diluted urine gives this reaction we may consider the presence of large quantities of carbohydrates. In these cases we get more positive results by using other tests. This test requires great cleanliness, and it has this inconvenience, that it is very difficult to get sufficiently pure sulphuric acid, and sometimes indeed perfectly pure α-naphthol. Several investigators, such as v. UDRÁNSKY, LUTHER, ROOS, and TREUPEL,[1] have investigated this test in regard to its applicability as an approximate test for carbohydrates in the urine.

Quantitative Estimation of Sugar in the urine. The urine for such an estimation must first be tested for proteid, and if any be present it must be removed by coagulation and the addition of acetic acid, care being taken not to increase or diminish the original volume of urine. The quantity of sugar may be determined by TITRATION with FEHLING'S or KNAPP'S solution, by FERMENTATION, or by POLARIZATION.

The titration liquids not only react with sugar, but also with certain other reducing substances, and on this account the titration methods give rather high results. When large quantities of sugar are present, as in typical diabetic urine, which generally contains a lower percentage of normal reducing constituents, this is indeed of little account; but when small quantities of sugar are present in an otherwise normal urine, the mistake may, on the contrary, be important, as the reducing power of normal urine may correspond to 5 p. m. grape-sugar (see page 506). In such cases the titration method must be employed in connection with the fermentation method, which will be described later. It is to be remarked that in typical diabetic urines with considerable quantities of sugar the titration with FEHLING'S solution is just as reliable as with KNAPP'S solution. When the urine, on the contrary, contains only little sugar with normal amounts of physiological constituents, then the titration with FEHLING's solution is more difficult, indeed in certain cases almost impossible, the results being very uncertain. In such cases KNAPP's method gives good results, according to WORM MÜLLER and his pupils.[1]

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[1] See Roos and Treupel, Zeitschr. f. physiol. Chem., Bdd. 15 u. 16.

The TITRATION with FEHLING'S SOLUTION depends on the power of sugar to reduce copper oxide in alkaline solutions. For this we formerly employed a solution which contained a mixture of copper sulphate, Rochelle salt, and sodium or potassium hydrate (FEHLING's solution); but as such a solution readily changes, we now prepare a copper-sulphate solution and an alkaline Rochelle-salt solution separately, and mix equal volumes of the two solutions before using.

The concentration of the copper-sulphate solution is such that 10 c. c. of this solution is reduced by 0.05 grm. grape-sugar. The copper-sulphate solution contains 34.65 grms. pure, crystallized, non-efflorescent copper sulphate in 1 litre. The sulphate is crystallized from a hot saturated solution by cooling and stirring; and the crystals are separated from the mother-liquor and pressed between blotting-paper until dry. The Rochelle-salt solution is prepared by dissolving 173 grms. of the salt in 350 c. c. water, adding 600 c. c. of a caustic-soda solution of a specific gravity of 1.12, and diluting with water to 1 litre. According to WORM MÜLLER, these three liquids—Rochelle-salt solution, caustic soda, and water—should be separately boiled before mixing together. For each titration mix in a small flask or porcelain dish exactly 10 c. c. of the copper-sulphate solution and 10 c. c. of the alkaline Rochelle-salt solution and add 30 c. c. water.

The urine free from proteid is diluted before the titration with water so that 10 c. c. of the copper solution requires between 5 and 10 c. c. of the diluted urine, which corresponds to between 1 and ½% sugar. A urine of a specific gravity of 1.030 may be diluted five times; one more concentrated, ten times. The urine so diluted is poured into a burette and allowed to flow into the boiling copper-sulphate and Rochelle-salt solution until the copper oxide is completely reduced. This has taken place when, immediately after boiling, the blue color of the solution disappears. It is very difficult and requires some practice to exactly determine this point, especially when the copper suboxide settles with difficulty. To determine whether the color has disappeared, allow the copper suboxide to settle a little below the meniscus formed by the surface of the liquid. If this layer is not blue, the operation is repeated, adding 0.1 c. c. less of urine; and if, after the copper suboxide has settled, the liquid has a blue color, the titration may be considered as completed. Because of the difficulty in obtaining this point exactly another end-reaction has been suggested. This consists in filtering immediately after boiling a small portion of the treated urine through a small filter into a test-tube which contains a little acetic acid and a few drops of potassium-ferrocyanide solution and water. The smallest quantity of copper is shown by a red coloration. If the operation is quickly conducted so that no oxidation of the suboxide into oxide takes place, this end-reaction is of value for urines which are rich in sugar and poor in urea and which have been strongly diluted with water. In urines poor in sugar which contain the normal amount of urea and which have not been strongly diluted, a rather abundant formation of ammonia from the urea may take place on boiling the alkaline liquid. This ammonia dissolves the suboxide in part, which easily passes into oxide thereby, and besides this the dissolved suboxide gives a red color with potassium ferrocyanide. In just those cases in which the titration is most difficult this end-reaction is the least reliable. Practice also renders it unnecessary, and it is therefore best to depend simply upon the appearance of the liquid.

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[1] Pflüger's Arch., Bdd. 16 u. 23; Journal f. prakt. Chem. (N. F.), Bd 26.

To facilitate the settling of the copper suboxide and thereby clearing the liquid, MUNK[1] has lately suggested the addition of a little calcium-chloride solution and boiling again. A precipitate of calcium tartrate is produced which carries down the suspended copper suboxide with it, and the color of the liquid can then be better seen. This artifice succeeds in many cases, but unfortunately there are urines in which the titration with FEHLING's solution in no way gives exact results. In those cases in which only small quantities of sugar exist in a urine rich in physiological constituents it is best to dissolve a very exactly weighed quantity of pure dextrose or dextrose-sodium chloride in the urine. The urine can now be strongly diluted with water and the titration is successful. The difference between the added sugar and that found by titration gives the reducing power of the original urine calculated as dextrose.

The necessary conditions for the success of the titration under all circumstances are, according to SOXHLET,[2] the following: The copper-sulphate and Rochelle-salt solution must, as above, be diluted to 50 c. c. with water; the urine must only contain between 0.5% and 1% sugar, and the total quantity of urine required for the reduction must be added to the titration liquid at once and boiled with it. From this last condition it follows that the titration is dependent upon minute details, and several titrations are required for each determination.

It is best to give here an example of the titration. The proper amount of copper-sulphate and Rochelle-salt solution and water (total volume = 50 c.c.) is heated to boiling in a flask; the color must remain blue. The urine diluted five times is now added to the boiling-hot liquid, 1 c. c. at a time; after each addition of urine boil for a few seconds, and look for the appearance of the end-reaction. If you find, for example, that 3 c. c. is too little, but that 4 c. c. is too much (the liquid becoming yellowish), then the urine has not been sufficiently diluted, for it should require between 5 and 10 c. c. of the urine to produce the complete reduction. The urine is now diluted ten times, and it should require between 6 and 8 c. c. for a total reduction. Now prepare four new tests, which are boiled simultaneously to save time, and add at one time respectively 6, 6½, 7, and 7½ c. c. of urine. If it is found that between 6½ and 7 c. c. are necessary to produce the end-reaction, then make four other tests, to which add respectively 6.6, 6.7, 6.8, and 6.9 c. c. of urine. If in this case the liquid is still somewhat bluish with 6.7 c. c. and completely decolorized with 6.8 c. c., we then consider the average figure 6.75 c. c. as correct.

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[1] Virchow's Arch., Bd. 105.

[2] Journal f. prakt. Chem. (N. F.), Bd. 21.

The calculation is simple. The 6.75 c. c. used contain 0.05 grm. sugar, and the percentage of sugar in the dilute urine is therefore $(6.75 : 0.05 = 100 : x) = \frac{5}{6.75} = 0.74$. But as the urine was diluted with ten times its volume of water, the undiluted urine contained $\frac{5 \times 10}{6.75} = 7.4\%$. The general formula on using 10 c. c. copper-sulphate solution is therefore $\frac{5 \times n}{k}$, in which n represents the number of times the urine has been diluted and k the number of c. c. used for the titration of the diluted urine.

The TITRATION ACCORDING TO KNAPP depends on the fact that mercuric cyanide is reduced into metallic mercury by grape-sugar. The titration liquid should contain 10 grms. chemically pure dry mercuric cyanide and 100 c. c. caustic-soda solution of a specific gravity of 1.145 per litre. When the titration is performed as described below (according to WORM MÜLLER and OTTO), 20 c. c. of this solution should correspond to exactly 0.05 grm. grape-sugar. If we proceed in other ways, the value of the solution is different.

Also in this titration the quantity of sugar in the urine should be between ½% and 1%, and here also the extent of dilution necessary must be determined by a preliminary test. To determine the end-reaction as described below, the test for excess of mercury is made with sulphuretted hydrogen.

In performing the titration allow 20 c. c. of KNAPP's solution to flow into a flask and dilute with 80 c. c. water, or, when you have reason to think that the urine contains less than 0.5% of sugar, only with 40–60 c. c. After this heat to boiling and allow the dilute urine to flow gradually into the hot solution, at first 2 c. c., then 1 c. c., then 0.5 c. c., then 0.2 c. c., and lastly 0.1 c. c. After each addition let it boil ½ minute. When the end-reaction is approaching, the liquid begins to clarify and the mercury separates with the phosphates. The end-reaction is determined by taking a drop of the upper layer of the liquid into a capillary tube and then blowing it out on pure white filter-paper. The moist spot is first held over a bottle containing fuming hydrochloric acid and then over strong sulphuretted hydrogen. The presence of a minimum quantity of mercury-salt in the liquid is shown by the spot becoming yellowish, which is seen best when it is compared with a second spot which has not been exposed to sulphuretted hydrogen. The end-reaction is still clearer when a small part of the liquid is filtered, acidified with acetic acid, and tested with sulphuretted hydrogen (OTTO[1]). The calculations are just as simple as for the previous method.

This titration, unlike the previous one, may be performed not only in daylight, but also in artificial light. KNAPP's method has the following advantages over FEHLING's method: It is applicable even when the quantity of sugar in the urine is very small and the quantity of the other urinary constituents is normal. It is more easily performed, and the titration liquids may be kept without decomposing for a long time (WORM MÜLLER and his pupils[2]). The views of different investigators on the value of this titration method are still somewhat contradictory.

ESTIMATION OF THE QUANTITY OF SUGAR BY FERMENTATION. This may be done in various ways; the simplest, and one at the same time sufficiently exact for ordinary cases, is ROBERTS'[3] method. This method consists in determining the specific gravity of the urine before and after fermentation. In the fermentation of sugar, carbon dioxide and alcohol are formed as chief products and the specific gravity is lowered, partly on account of the disappearance of the sugar and partly on account of the production of alcohol. ROBERTS found that a decrease of 0.001 in the specific gravity corresponded to 0.23% sugar, and this has been substantiated since by several other investigators (WORM MÜLLER[4] and others). If the urine, for example, has a specific gravity of 1.030 before fermentation and 1.008 after, then the quantity of sugar contained therein was 22 × 0.23 = 5.06%.

In performing this test the specific gravity must be taken at the same temperature before and after the fermentation. The urine must be faintly acid, and when necessary it should be acidified with a little tartaric-acid solution. The activity of the yeast must, when necessary, be controlled by a special test. Place 200 c. c. of the urine in a 400-c. c. flask and add a piece of compressed yeast the size of a pea, and subdivide the yeast through the liquid by shaking, close the flask with a stopper provided with a finely drawn-out glass tube, and allow the test to stand at the temperature of the room or, still better, at + 20–25° C. After 24–48 hours the fermentation is ordinarily ended, but this must be verified by the bismuth test. After complete fermentation filter through a dry filter, bring the filtrate to the proper temperature, and determine the specific gravity.

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[1] Journal f. prakt. Chem., Bd. 26.

[2] Pflüger's Arch., Bdd. 16 u. 23.

[3] Edinburgh Med. Journal, Oct. 1861; The Lancet, Vol. 1, 1862.

[4] Pflüger's Arch., Bdd. 33 u. 37.

If the specific gravity be determined with a good pyknometer supplied with a thermometer and an expansion-tube, this method, when the quantity of sugar is not less than 4–5 p. m., gives, according to WORM MÜLLER, very exact results, but this has been disputed by BUDDE.[1] For the physician the method in this form is not quite serviceable. Even when the specific gravity is determined by a delicate urinometer which can give the density to the fourth decimal, we do not obtain quite exact results, because of the principal errors of the method (BUDDE); but the errors are usually smaller than those which occur in titrations made by unpractised hands. Among the methods proposed and closely tested for the quantitative estimation of sugar, we have none which are at the same time easily performed and which give positive results in other than experienced hands.

When the quantity of sugar is less than 5 p. m. these methods cannot be used. Such a small quantity of sugar cannot, as above mentioned, be determined by titration directly, because the reduction power of normal urine corresponds to 4–5 p. m. In such cases, according to WORM MÜLLER, first determine the reduction power of the urine by titration with KNAPP's solution, then ferment the urine with the addition of yeast, and titrate again with KNAPP's solution. The difference found between the two titrations calculated as sugar gives the true quantity of sugar.

ESTIMATION OF SUGAR BY POLARIZATION. In this method the urine must be clear, not too deeply colored, and, above all, must not contain any other optically active substances besides glucose. By using a delicate instrument and with sufficient practice very exact results can be obtained by this method. For the physician, ROBERTS' fermentation test, which requires no expensive apparatus and no special practice, is to be preferred. Under such circumstances, and as the estimation by means of polarization can be performed with exactitude only by specially instructed chemists, it is hardly necessary to give this method in detail, and the reader is referred to special works for instructions in the use of the apparatus.

Levulose. Lævogyrate urines containing sugar have been observed by VENTZKE, ZIMMER and CZAPEK, SEEGEN, and others.[2] The nature of the substance causing this action is difficult to describe exactly, but there is hardly any doubt that the urine, at least in certain cases, as in those observed by SEEGEN, contains levulose. The occurrence of lævulose in the urine from SEEGEN's patient has been made very possible by KÜLZ.[3]

The presence of levulose in a urine containing sugar is only probable when the urine is lævogyrate or optically inactive, or when it shows a reduction power not corresponding (less) to the dextrorotary power, or when it contains no other lævœgyrate substance (β-oxybutyric acid, conjugated glycuronic acids, protein bodies, or cystin). Levulose ferments with yeast and yields the same osazone as glucose.

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[1] Ugeskrift for Laeger. (4), Bd. 9; Pflüger's Arch., Bd. 40; Zeitschr. f. physicl. Chem., Bd. 13.

[2] See Huppert-Neubauer, Harnanalyse, 10. Aufl., S. 125.

[3] Zeitschr. f. Biologie, Bd. 27.

Laiose is a substance found by Leo[1] in diabetic urines in certain cases, and which Leo considers as a sugar. It is lævœgyrate, amorphous, and has no sweet taste, but rather a sharp and salty taste. Laiose has a reducing action on metallic oxides, does not ferment, and gives a non-crystalline, yellowish-brown oil with phenylhydrazin. We have no positive proof as yet that this substance is a sugar.

MILK-SUGAR. The appearance of milk-sugar in the urine with engorgement of milk has been made known especially by the investigations of De Sinety and F. Hofmeister.[2] After taking large quantities of milk-sugar some lactose may be found in the urine (see Chapter IX on absorption).

The positive detection of milk-sugar in the urine is difficult, because this sugar is, like glucose, dextrogyrate and also gives the usual reduction tests. If urine contains a dextrogyrate, non-fermentable sugar which reduces bismuth solutions, then it is very probable that it contains milk-sugar. It must be remarked that the fermentation test for milk-sugar is, according to the experience of Lusk and Voit,[3] best performed by using pure cultivated yeast (saccharomyces apiculatus). This yeast only ferments the glucose, while it does not decompose the milk-sugar. The most positive means for the detection of lactose is to isolate the sugar from the urine. This may be done by the following method, suggested by F. Hofmeister:[4]

Precipitate the urine with sugar of lead, filter, wash with water, unite the filtrate and wash-water, and precipitate with ammonia. The liquid filtered from the precipitate is again precipitated by sugar of lead and ammonia until the last filtrate is optically inactive. The several precipitates with the exception of the first, which contains no sugar, are united and washed with water. The washed precipitate is decomposed in the cold with sulphuretted hydrogen and filtered. The excess of sulphuretted hydrogen is driven off by a current of air; the acids set free are removed by shaking with silver oxide. Now filter, remove the dissolved silver by sulphuretted hydrogen, treat with barium carbonate to unite with any free acetic acid present, and concentrate. Before the evaporated residue is syrupy it is treated with 90% alcohol until a flocculent precipitate is formed which settles quickly. The filtrate from this when placed in a desiccator deposits crystals of milk-sugar, which are purified by recrystallization, decolorizing with animal charcoal and boiling with 60–70% alcohol.

Pentoses. Salkowski and Jastrowitz[5] found in the urine of persons addicted to the morphin habit a variety of sugar which was a pentose and yielded an osazone which melted at 159° C. SALKOWSKI[1] has observed two new cases of pentosuria. The pentose in the urine seemed to be identical with the pentose obtained by HAMMARSTEN on the cleavage of a pancreas proteid. E. KÜLZ and J. VOGEL[2] have detected pentoses in the urine of diabetics, as well as in that of dogs with pancreas or phlorhizin diabetes. Concentrated hydrochloric acid saturated with phloroglucin may be used in detecting pentoses. Add ½ volume of the urine to be tested to the acid and warm. In the presence of pentoses the red coloration mentioned on page 65 appears. This test is not conclusive, as glycuronic acid gives the same reaction; further investigation is therefore necessary.

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[1] Virchow's Arch., Bd. 107.

[2] Zeitschr. f. physiol. Chem. Bd. 1, S. 101, which also contains the pertinent literature.

[3] Carl Voit, über die Glycogenbildung nach Aufnahme verschiedener Zuckerarten, Zeitschr. f. Biologie, Bd. 28.

[4] L. c.

[5] Centralbl. f. d. med. Wissensch., 1892, Nos. 19 and 32.

INOSIT occurs only rarely, and in but small quantities, in the urine in albuminuria and in diabetes mellitus. After excessive drinking of water inosit is found in the urine. According to HOPPE-SEYLER[3] traces of inosit occur in all normal urines.

In detecting inosit the proteid is first removed from the urine. Then concentrate the urine on the water-bath to ¼ and precipitate with sugar of lead. The filtrate is warmed and treated with basic lead acetate as long as a precipitate is formed. The precipitate formed after 24 hours is washed with water, suspended in water, and decomposed with sulphuretted hydrogen. A little uric acid may separate from the filtrate after a short time. The liquid is filtered, concentrated to a syrupy consistency, and treated while boiling with 3–4 vols. alcohol. The precipitate is quickly separated. After the addition of ether to the cooled filtrate, crystals separate after a time, and these are purified by decolorization and recrystallization. With these crystals perform the tests mentioned on page 370.

Acetone and **Diacetic Acid.** These bodies, the occurrence in the urine and formation in the organism of which have been the subject of numerous investigations, especially by v. JAKSCH,[4] were first observed in urine in diabetes mellitus (PETERS, KAULICH, v. JAKSCH, GERHARDT). Acetone may give the diabetic urine as well as the expired air the odor of apples or other fruit. According to v. JAKSCH and others acetone is a normal urinary constituent, though it may only occur in very small amounts (0.01 grm. in the 24 hours).

Acetone may, as found by v. JAKSCH, be a by-product in lactic-acid fermentation, and this origin for the traces of acetone eliminated by the normal urine requires further proof. There is no doubt that the appearance of acetone as well as diacetic acid is essentially caused by an increased destruction of proteid. This follows from the very marked increase in the elimination of acetone and diacetic acid during inanition (v. JAKSCK,[1] FR. MÜLLER[2]). This is also in accord with the observations of WRIGHT[3] that in diabetes no relationship exists between the elimination of acetone and sugar, while there is a relationship between the elimination of acetone and nitrogen; thus on the days when most nitrogen is eliminated we find the highest results for the acetone, and *vice versa*. Abundant proteid food also increases the elimination of acetone, according to HONIGMANN[4] and v. NOORDEN,[5] apparently in the case where with a one-sided proteid food an insufficient supply of calories takes place, which leads to a reduction of the body-proteid. According to this view, which requires further proof, the extent of the elimination of acetone and diacetic acid is not dependent upon the extent of the metabolism of proteid, but upon the quantity of destroyed body-proteid.

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[1] Berliner klin. Wochenschr., 1895.

[2] Zeitschr. f. Biologie, Bd. 32.

[3] Handbuch d. physiol. u. pathol. chem. Analyse, 6. Aufl., S. 196.

[4] In regard to the extensive literature on acetone and diacetic acid we refer the reader to Huppert-Neubauer, Harnanalyse, 10. Aufl., and v. Noorden's Lehrb. d. Pathol. des Stoffwechsels. Berlin, 1893.

According to this view it is also clear that an abundant elimination of acetone and diacetic acid is observed, especially in such diseases in which an abundant destruction of body-proteid takes place, such as fevers, diabetes, disturbed digestion, mental debility with abstinence, cachexia, etc. It has not been proven how far the acetonuria experimentally produced by LUSTIG[6] by lesion of the sinus fovea rhomboidalis or by excision of the solar plexus is caused by the generally disturbed condition of the animal or by other circumstances.

Diacetic acid has not been observed as a physiological constituent of the urine. It occurs in the urine chiefly under the same conditions as acetone; still we have cases in which only acetone and no diacetic acid appears. Like acetone the diacetic acid occurs often in children, especially in high fevers, acute exanthema, etc. Diacetic acid decomposes readily into acetone. According to ARAKI[7] it is probably produced as an intermediate product in the oxidation of β-oxybutyric acid in the organism. The three bodies appearing in the urine, acetone, diacetic acid, and oxybutyric acid, stand in close relationship to each other.

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[1] Ueber Acetonurie und Diaceturie. Berlin, 1885.

[2] Bericht über die Ergebnisse des an Cetti ausgeführten Hungerversuches. Berlin. klin. Wochenschr., 1887.

[3] See Maly's Jahresber., Bd. 21, S. 404.

[4] Zur Enstehung des Acetons. Diss. Breslau, 1886. Cited from v. Noorden, Lehrb., S. 177.

[5] L. c., S. 78.

[6] Centralbl. f. Physiol., Bd. 6.

[7] Zeitschr. f. physiol. Chem., Bd. 18.

Acetone, dimethyl ketone, C_3H_6O or $CO.(CH_3)_2$, is a thin water-clear liquid boiling at 56.5° C. and with a pleasant odor of fruit. It is lighter than water, with which it mixes in all proportions, also with alcohol and ether. The most important reactions for acetone are the following:

LIEBEN'S[1] *Iodoform Test.* When a watery solution of acetone is treated with alkali and then with some iodine-potassium-iodide solution and gently warmed a yellow precipitate of iodoform is formed, which is known by its odor and by the appearance of the crystals (six-sided plates or stars) under the microscope. This reaction is very delicate, but it is not characteristic of acetone. GUNNING'S[2] *modification of the iodoform test* consists in using an alcoholic solution of iodine and ammonia instead of the iodine dissolved in potassium iodide and alkali hydrate. In this case, besides iodoform, a black precipitate of iodide of nitrogen is formed, but this gradually disappears on standing, leaving the iodoform visible. This modification has the advantage that it does not give any iodoform with alcohol. On the other hand, it is not quite so delicate, but still it detects 0.01 milligramme acetone in 1 c. c.

REYNOLD'S[3] *mercuric-oxide test* is based on the power of acetone to dissolve freshly precipitated HgO. A mercuric-chloride solution is precipitated by alcoholic caustic potash. To this add the liquid to be tested for acetone, shake well and filter. In the presence of acetone the filtrate contains mercury, which may be detected by ammonium sulphide. This test has about the same delicacy as GUNNING'S test.

LEGAL'S[4] *Sodium-nitroprusside Test.* If an acetone solution is treated with a few drops of a freshly prepared sodium-nitroprusside solution and then with caustic-potash or soda solution, the liquid is colored ruby-red. Creatinin gives the same color; but if we saturate with acetic acid, the color becomes carmine or purplish red in the presence of acetone, but yellow and then gradually green and blue in the presence of creatinin. If we use ammonia instead of the caustic alkali (LE NOBEL), the reaction takes place with acetone, but not with creatinin.[1] LEGAL's test indicates even 0.1 milligrm. acetone.

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[1] Annal. d. Chem. u. pharm., Suppl. Bd. 7.

[2] Gunning, by Bardy, Journ. de pharm. et chim. (5), Tome 4.

[3] Cited from Huppert-Neubauer, Harnanalyse, 10. Aufl., S. 60.

[4] Breslauer ärztl. Zeitschr., 1883.

PENZOLDT's[2] *indigo test* depends on the fact that orthonitrobenzaldehyde in alkaline solution with acetone yields indigo. A warm saturated and then cooled solution of the aldehyde is treated with the liquid to be tested for acetone and then with caustic soda. In the presence of acetone the liquid first becomes yellow, then green, and lastly indigo separates; and this may be dissolved with a blue color by shaking with chloroform. 1.6 milligrms. acetone can be detected by this test.

MALERBA[3] uses a solution of dimethylparaphenylendiamin as a reagent for acetone. It gives a red liquid which has an absorption-spectrum very similar to oxyhæmoglobin.

Diacetic acid, or aceto-acetic acid, $C_4H_6O_3$ or $C_2H_3O.CH_2.COOH$. This acid is a colorless, strongly acid liquid which mixes with water, alcohol, and ether in all proportions. On heating to boiling with water, and especially with acids, this acid decomposes into carbon dioxide and acetone, and therefore gives the above-mentioned reactions for acetone. It differs from acetone in that it gives a violet-red or brownish-red color with a dilute ferric-chloride solution. This color decreases even at the ordinary temperature within 24 hours, and more quickly on boiling. It differs in this from phenol, salicylic acid, acetic acid, or sulphocyanides.

Detection of Acetone and Diacetic Acid in the urine. Before testing for acetone test for diacetic acid, and as this acid gradually decomposes on allowing the urine to stand, the urine must be as fresh as possible. In the presence of diacetic acid the urine gives the so-called GERHARDT's reaction, showing a wine-red color on the addition of a dilute, not too acid, ferric-chloride solution. Treat 10–50 c. c. of the urine with ferric chloride as long as it gives a precipitate, filter the precipitate of ferric phosphate, and add some more ferric chloride to the filtrate. In the presence of the acid a claret-red color is produced. After this heat a second, similar portion of the faintly acid urine to boiling, and repeat the test on cooling, which should now give negative results. A third portion of urine is acidified with sulphuric acid and shaken with ether (which takes up the acid). Now shake the removed ether with a very dilute watery solution of ferric chloride, and the watery layer becomes violet-red or claret-red. The color disappears on warming.

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[1] According to the author this statement is not correct.

[2] Arch. f. klin. Med., Bd. 34.

[3] Atti della R. Academ. med. chirurg. di Napoli, Anno 48, Nuova Serie,

K. MÖRNER[1] suggests that in testing for diacetic acid in the urine the urine be treated with a little KI and Fe_2Cl_6 in excess and heated. In the presence of diacetic acid very irritating vapors of iodoacetone (?) are developed.

In the absence of diacetic acid the acetone may be tested for directly. This may be done directly on the urine by PENZOLDT's test. This test, which is only approximate, is only of value when the urine contains a considerable amount of acetone. For a more accurate test we distil at least 250 c. c. of the urine faintly acidified with sulphuric acid, care being taken to have a good condensation. Most of the acetone is contained in the first 10–20 c. c. of the distillate. This distillate is tested for acetone by the above tests. In testing for acetone in the simultaneous presence of diacetic acid, first make the urine faintly alkaline, and shake it carefully with ether free from alcohol and acetone in a separatory funnel. The removed ether is then shaken with water, which takes up the acetone, and then the watery liquid is tested.

The quantitative estimation of acetone in the urine is done by converting it first into iodoform. The urine is acidified with acetic acid (according to HUPPERT, 1–2 c. c. 50% acetic acid for every 100 c. c. urine) and distilled. The iodoform formed is determined in the distillate either gravimetrically according to KRÄMER or colorimetrically, according to v. JAKSCH. It is best to proceed according to the method as suggested by MESSINGER and HUPPERT.[2] They determine the quantity of acetone by determining the quantity of iodine necessary in the formation of iodoform by titration. In regard to this method and its execution we refer the reader to HUPPERT-NEUBAUER.[3]

β-Oxybutyric Acid, $C_4H_8O_3$ or $CH_3.CH(OH).CH_2COOH$. The appearance of this acid in the urine was first positively shown by MINKOWSKI,[4] KÜLZ[5] and STADELMANN.[6] It occurs especially in difficult cases of diabetes, but it has also been observed in scarlet fever and in measles (KÜLZ), in scurvy (MINKOWSKI), and in diseases of the brain with abstinence (KÜLZ). β-oxybutyric acid is undoubtedly derived from an abnormal destruction of body-proteid, and it therefore occurs in the urine in inanition, cachexia, etc. β-oxybutyric acid is accompanied by diacetic acid in the urine, while on the other hand the last-mentioned acid occurs in the urine without the first.

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[1] Skan. Arch. f. Physiol., Bd. 5.

[2] Huppert-Neubauer, Harnanalyse, 10. Aufl., S. 760, which also contains the description of other methods and summary of the literature.

[3] L. c., 10. Aufl.

[4] Arch. f. exp. Path. u. Pharm., Bd. 18 u. 19.

[5] Zeitschr. f. Biologie, Bdd. 20 u. 23.

[6] Arch. f. exp. Path. u. Pharm., Bd. 17.

β-oxybutyric acid forms an odorless syrup which mixes readily with water, alcohol, and ether. This acid is optically active and indeed lævogyrate, and it therefore interferes with the estimation of sugar in the urine by means of polarization. It is not precipitated either by basic lead acetate or by ammoniacal basic lead acetate. On boiling with water, especially in the presence of a mineral acid, this acid decomposes into α-CROTONIC ACID, which melts at 71–72° C., and water: $CH_3.CH(OH).CH_2.COOH = H_2O + CH_3.CH:CH.COOH$. It yields acetone on oxidation with a chromic-acid mixture.

Detection of β-Oxybutyric Acid in the urine. If a urine is still lævogyrate after fermentation with yeast, the presence of oxybutyric acid is probable. A further test may be made, according to KÜLZ, by evaporatingthe fermented urine to a syrup, and, after the addition of an equal volume of concentrated sulphuric acid, distilling directly without cooling. α-crotonic acid is produced which distils over, and, after collecting in a test-tube, crystals, which melt at + 72° C., separate on strongly cooling. If no crystals are obtained, then shake the distillate with ether, and test the melting-point of the residue obtained after evaporating the ether which has been washed with the water. According to MINKOWSKI the acid may be isolated as a silver-salt.[1]

EHRLICH'S[2] *Urine Test.* Mix 250 c. c. of a solution which contains 50 c. c. HCl and 1 grm. sulphanilic acid in one litre with 5 c. c. of a $\frac{1}{2}$% solution of sodium nitrite (which produces very little of the active body, sulphodiazobenzol) In performing this test treat the urine with an equal volume of this mixture and then supersaturate with ammonia. Normal urine will become yellow thereby, or orange after the addition of ammonia (aromatic oxyacids may sometimes give after a certain time red azo bodies which color the upper layer of phosphate sediment). In pathological urines we sometimes have (and this is the characteristic diazo reaction) a primary yellow coloration, with a very marked secondary red coloration on the addition of ammonia, and the froth is also tinged with red. The upper layer of the sediment becomes greenish. The body which gives this reaction is unknown, but it occurs especially in the urine of typhoid patients (EHRLICH). Views are divided in regard to the significance of this reaction.

ROSENBACH'S urine test, which consists in adding nitric acid drop by drop to the boiling-hot urine and obtaining a claret-red coloration and a bluish-red foam on shaking, depends upon the formation of indigo substances, especially indigo red.[3]

FAT in the urine. The elimination of a urine which in appearance and richness in fat resembles chyle is called *chyluria.* It contains habitually proteid, and often fibrin. Chyluria occurs mostly in the inhabitants of the tropics. *Lipuria*, or the elimination of fat with the urine, may appear in apparently healthy persons, sometimes with and sometimes without albuminuria, in pregnancy, and also in certain diseases, as in diabetes, poisoning with phosphorus, and fatty degeneration of the kidneys.

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[1] Arch. f. exp. Path. u. Pharm., Bd. 18, S. 35; Zeitschr. f. anal. Chem., Bd. 24, S. 153.

[2] Zeitschr. f. klin. Med., Bd. 5.

[3] See Rosin, Virchow's Arch., Bd. 123.

Fat is usually detected by the microscope. It may also be dissolved with ether, and it may always be detected by evaporating the urine to dryness and extracting the residue with ether.

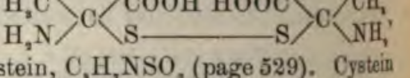
CHOLESTERIN is also sometimes found in the urine in chyluria and in a few other cases.

LEUCIN AND TYROSIN. These bodies are found in the urine, especially in acute yellow atrophy of the liver, in acute phosphorus-poisoning, and in difficult cases of typhoid and smallpox.

Detection of Leucin and Tyrosin. Tyrosin occurring as sediment may be identified by means of the microscope; but if a positive proof is desired, a recrystallization of the same from ammonia or ammoniacal alcohol is necessary.

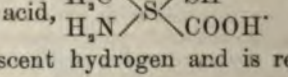
To detect both these bodies when they occur in solution in the urine, proceed in the following manner: The urine free from proteid is precipitated by basic lead acetate, the lead removed from the filtrate by H_2S, and concentrated as much as possible. The residue is extracted with a small quantity of absolute alcohol to remove the urea. The residue is then boiled with faintly ammoniacal alcohol, filtered, the filtrate evaporated to a small volume and allowed to crystallize. If no tyrosin crystals are obtained, then dilute with water, precipitate again with basic lead acetate, and proceed as before. If tyrosin crystals now separate, they are filtered, and the filtrate still further concentrated to obtain the leucin crystals.

Cystin $(C_3H_6NSO_2)_2$. This body is, according to BAUMANN,[1] to be considered as disulphide,



$$\left.\begin{matrix}H_3C\\H_2N\end{matrix}\right>C\left<\begin{matrix}COOH & HOOC\\S & \!\!\!\text{———}\!\!\! S\end{matrix}\right>C\left<\begin{matrix}CH_3\\NH_2\end{matrix}\right.,$$

of the previously mentioned cystein, $C_3H_7NSO_2$ (page 529). Cystein itself is α-amidothiolactic acid,



$$\left.\begin{matrix}H_3C\\H_2N\end{matrix}\right>S\left<\begin{matrix}SH\\COOH\end{matrix}\right..$$

Cystin is converted into cystein by nascent hydrogen and is reconverted into cystin by oxidation.

BAUMANN and GOLDMANN[2] claim that a substance similar to cystin occurs in very small amounts in normal urine. This substance occurs in large quantities in the urine of dogs after poisoning with phosphorus. Cystin itself is found with positiveness, and even then very rarely, only in urinary calculi and in pathological urines, from which it may separate as a sediment. Cystinuria occurs oftener in men than in women, and cystin seems to be an abnormal splitting product of the proteids. BAUMANN and v. UDRÁNSZKY[3] found in urine in cystinuria the two diamins, *cadaverin* (pentamethylendiamin) and *putrescin* (tetramethylendiamin), which are produced in the putrefaction of proteids. These two diamins were also found in the contents of the intestine in cystinuria, while under normal conditions they are not present. The Author therefore considers that perhaps some connection exists between the formation of diamins in the intestine, by the peculiar putrefaction in cystinuria, and cystinuria itself. Cadaverin has also been found in the urine in cystinuria by Stadthagen and Brieger.[1] Cystin has also been found in ox-kidneys, in horse's liver (Drechsel[2]), and as traces in the liver of a drunkard. Külz[3] once observed the occurrence of cystin during the digestion of fibrin with pancreas.

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[1] Zeitschr. f. Physiol. Chem., Bd. 8. In regard to the literature on cystin see Brenzinger, *ibid.*, Bd. 16, S. 552.

[2] Zeitschr. f physiol. Chem., Bd. 12.

[3] *Ibid.*, Bd. 13

Cystin crystallizes in thin, colorless, six-sided plates. It is not soluble either in water, alcohol, ether, or acetic acid, but dissolves in mineral acids and oxalic acid. It also dissolves in alkalies and in ammonia, but not in ammonium carbonate. Cystin is optically active and strongly lævorotatory. If cystin is boiled with caustic alkali it decomposes, yielding among other products alkali sulphides, which may be detected by lead acetate or sodium nitroprusside. On treating cystin with tin and hydrochloric acid, only a little sulphuretted hydrogen is evolved and cystein is produced. On shaking a solution of cystin in an excess of caustic soda with benzoylchloride a voluminous precipitate of benzoyl-cystin is produced (Baumann and Goldmann[4]). On heating on platinum foil, cystin does not melt, but ignites and burns with a bluish-green flame accompanied by a peculiar sharp odor. On warming with nitric acid cystin dissolves with decomposition and leaves a reddish-brown residue on evaporation which does not give the murexid test.

Cystein hydrochloride gives a nearly insoluble precipitate having the composition $2(C_3H_7NSO_2) + 3HgCl_2$ with mercuric chloride. Baumann and Borissow[5] have based a method for the quantitative estimation of cystin on this behavior. They first reduce the cystin by zinc and hydrochloric acid.

Cystin is easily prepared from cystin calculi by dissolving them in alkali carbonate, precipitating the solution with acetic acid, and redissolving the precipitate in ammonia. The cystin crystallizes on the spontaneous evaporation of the ammonia. The cystin dissolved in the urine is detected, in the absence of proteid and sulphuretted hydrogen, by boiling with alkali and testing with lead salt or sodium nitroprusside. To isolate cystin from the urine, acidify the urine strongly with acetic acid. The precipitate containing cystin is collected after 24 hours and digested with hydrochloric acid, which dissolves the cystin and calcium oxalate, leaving the uric acid undissolved. Filter, supersaturate the filtrate with ammonium carbonate, and treat the precipitate with ammonia, which dissolves the cystin and leaves the calcium oxalate. Filter again and precipitate with acetic acid. The precipitated cystin is identified by the microscope and the above-mentioned reactions. Cystin as a sediment is identified by the microscope. It must be purified by dissolving in ammonia and precipitating with acetic acid and then tested. Traces of dissolved cystin may be detected by the production of benzoyl-cystin, according to BAUMANN and GOLDMANN.

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[1] Berl. klin. Wochenschr., 1889.

[2] Du Bois-Reymond's Arch., 1891.

[3] Zeitschr. f Biologie, Bd. 27.

[4] Zeitschr f. physiol Chem., Bd. 12.

[5] *Ibid.*, Bd. 19, S. 511.

VII. Urinary Sediments and Calculi.

Urinary sediment is the more or less abundant deposit which is found in the urine after standing. This deposit may consist partly of organized and partly of non-organized constituents. The first, consisting of cells of various kinds, yeast-fungus, bacteria, spermatozoa, casts, etc., must be investigated by means of the microscope, and the following only applies to the non-organized deposits.

As above mentioned (page 447), the urine of healthy individuals may sometimes, even on voiding, be cloudy on account of the phosphates present, or become so after a little while because of the separation of urates. As a rule, urine just voided is clear, and after cooling shows only a faint cloud (nubecula), which consists of so-called mucus, a few epithelium-cells, mucous corpuscles, and urate particles. If an acid urine is allowed to stand, it will gradually change; it becomes darker and deposits a sediment consisting of uric acid or urates, and sometimes also calcium-oxalate crystals, in which yeast-fungus and bacteria are often to be seen. The cause of this change, which the earlier investigators called "ACID FERMENTATION OF THE URINE," is, according to SCHERER,[1] the mucus, which acts like an enzyme or ferment, producing an acetic-acid or lactic-acid fermentation, precipitating free uric acid or acid urates. According to NEUBAUER,[2] an actual acid fermentation may occur in diabetic urine, but this seems to occur only very seldom, and according to RÖHMANN[1] an acid fermentation of the urine in SCHERER's sense does not occur under normal conditions. According to VOIT and HOFMANN[2] a separation of free uric acid and acid urates may be produced, without any increase in the acid reaction, by an exchange of the di-hydrogen alkali phosphates with the alkali urate on cooling and on standing. Simple acid phosphate and, according to the conditions, acid urate or free uric acid are formed. A gradual precipitation of uric acid may occur not only without an increase in the acid reaction, but, because of the alkaline reaction of the simple acid-alkali phosphate, it may occur with a simultaneous decrease of the same. RÖHMANN has presented objections to this statement. He claims that a steady decrease of the acid reaction, without formation of ammonia, caused by the above-mentioned transformation of phosphates and urates does not take place. The acid reaction does not decrease until the ammonia increases. According to BENCE JONES[3] the precipitation of the uric acid and urates has another cause. He claims that the urine contains hyperacid salts, so-called quadriurates (see page 478), which gradually split into uric acid and biurates.

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[1] Annal. d Chem. u. Pharm., Bd. 42 (1842).

[2] Neubauer und Vogel, Analyse des Harns (1876).

Earlier or later, sometimes only after several weeks, the reaction of the original acid urine changes and becomes neutral or alkaline. The urine has now passed into the "ALKALINE FERMENTATION," which consists in the decomposition of the urea into carbon dioxide and ammonia by means of lower organisms, micrococcus ureæ, bacteria ureæ, and other bacteria. MUSCULUS[4] has isolated an enzyme from the micrococcus ureæ which decomposes urea and is soluble in water. During the alkaline fermentation volatile fatty acids, especially acetic acid, may be produced, chiefly by the fermentation of the carbohydrates of the urine (SALKOWSKI[5]). A fermentation by which nitric acid is reduced to nitrous acid, and another where sulphuretted hydrogen is produced, may sometimes occur.

If the alkaline fermentation has only advanced so far as to render the reaction neutral, then we often find in the sediment fragments of uric-acid crystals, sometimes covered with prismatic crystals of alkali urate; dark-colored spheres of ammonium urate, often crystals of calcium oxalate, and sometimes crystallized calcium phosphate are also found. Crystals of ammonium-magnesium phosphate (triple phosphate) and spherical ammonium urate are specially characteristic of alkaline fermentation. The urine in alkaline fermentation becomes paler and is often covered with a fine membrane which contains amorphous calcium phosphate and glistening crystals of triple phosphate and numerous micro-organisms.

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[1] Zeitschr. f. physiol. Chem., Bd. 5.

[2] Sitzungsber. d. k. b. Akad. d. Wissensch., 1867, Bd. 2, S. 279. Cited from Röhmann, l. c.

[3] Journ. Chem. Soc., Vol. XV. p. 8.

[4] Pflüger's Arch., Bd. 12.

[5] Zeitschr. f. physiol. Chem., Bd. 13.

Non-organized Sediments.

Uric Acid. This acid occurs in acid urines as colored crystals which are identified partly by their form and partly by their property of giving the murexid test. On warming the urine they are not dissolved. On the addition of caustic alkali to the sediment the crystals dissolve, and when a drop of this solution is placed on a microscope-slide and treated with a drop of hydrochloric acid, small crystals of uric acid are obtained which are easily seen under the microscope.

Acid Urates. These only occur in the sediment of acid or neutral urines. They are amorphous, clay-yellow, brick-red, rose-colored, or brownish red. They differ from other sediments in that they dissolve on warming the urine. They give the murexid test, and small microscopic crystals of uric acid separate on the addition of hydrochloric acid. Crystalline alkali urates occur very rarely in the urine, and as a rule only in such as have become neutral but not alkaline by the alkaline fermentation. The crystals are somewhat similar to those of neutral calcium phosphate; they are not dissolved by acetic acid, however, but give a cloudiness therewith due to small crystals of uric acid.

Ammonium urate may indeed occur as a sediment in a neutral urine which at first was strongly acid and has become neutralized by the alkaline fermentation, but it is only characteristic of ammoniacal urines. This sediment consists of yellow or brownish, rounded spheres which are often covered with thorny-shaped prisms and, because of this, are rather large and resemble the thorn-apple. It gives the murexid test. It is dissolved by alkalies with the development of ammonia, and crystals of uric acid separate on the addition of hydrochloric acid to this solution.

Calcium oxalate occurs in the sediment generally as small, shining, strongly refractive quadratic octahedra, which on microscopical examination remind one of a letter-envelope. The crystals can only be mistaken for small, not fully developed crystals of ammonium-magnesium phosphate. They differ from these by their insolubility in acetic acid. The oxalate may also occur as flat, oval, or nearly circular disks with central cavities which from the side appear like an hour-glass. Calcium oxalate may occur as a sediment in an acid as well as in a neutral or alkaline urine. The quantity of calcium oxalate separated from the urine as sediment depends not only upon the amount of this salt present, but also upon the acidity of urine. The solvent for the oxalate in the urine seems to be the double-acid alkali phosphate, and the greater the quantity of this salt in the urine the greater the quantity of oxalate in solution. When, as above mentioned (page 565), the simple-acid phosphate is formed from the double-acid phosphate, on allowing the urine to stand, a corresponding part of the oxalate may be separated as sediment.

Calcium carbonate occurs in considerable quantities as sediment in the urine of herbivora. It occurs in but small quantities as a sediment in human urine, and in fact only in alkaline urines. It either has almost the same appearance as amorphous calcium oxalate, or it occurs as somewhat larger globules with concentric bands. It dissolves in acetic acid with the generation of gas, which differentiates it from calcium oxalate. It is not yellow or brown like ammonium urate, and does not give the murexid test.

Calcium sulphate occurs very rarely as a sediment in strongly acid urines. It appears as long, thin, colorless needles, or generally as plates grouped together.

Calcium Phosphate. The CALCIUM TRIPHOSPHATE, $Ca_3(PO_4)_2$, which occurs only in alkaline urines, is always amorphous and occurs partly as a colorless, very fine powder and partly as a membrane consisting of very fine granules. It differs from the amorphous urates in that it is colorless, dissolves in acetic acid, but remains undissolved on warming the urine. CALCIUM DIPHOSPHATE, $CaHPO_4 + 2H_2O$, occurs in neutral or only in very faintly acid urine. It is found sometimes as a thin film covering the urine, and sometimes as a sediment. In crystallizing the crystals may be single, or they may cross one another, or they may be arranged in groups of colorless, wedge-shaped crystals whose wide end is sharply defined. These crystals differ from crystalline alkaline urates in that they dissolve without a residue in dilute acids and do not give the murexid test.

Ammonium-magnesium phosphate, TRIPLE PHOSPHATE, may separate of course from an amphoteric urine in the presence of a sufficient quantity of ammonium salts, but it is generally characteristic of a urine become ammoniacal through alkaline fermentation. The crystals are so large that they may be seen with the unaided eye as colorless glistening particles in the sediment, on the walls of the vessel, and in the film on the surface of the urine. This salt forms large prismatic crystals of the rhombical system (coffin-shaped) which are easily soluble in acetic acid. Amorphous *magnesium triphosphate*, $Mg_3(PO_4)_2$, occurs with calcium triphosphate in urines rendered alkaline by a fixed alkali. Crystalline magnesium phosphate, $Mg_3(PO_4)_2 + 22H_2O$, has been observed in a few cases in human urine (also in horse's urine) as strongly refractive, long rhombical plates.

Kyestein is the film which appears after a little while on the surface of the urine. This coating, which was formerly considered as characteristic of urine in pregnancy, contains various elements, such as fungus, vibriones, epithelium-cells, etc. It often contains earthy phosphates and triple phosphate crystals.

As more rare sediments we find *cystin*, *tyrosin*, *hippuric acid*, *xanthin*, *hæmatoidin*. In alkaline urine blue crystals of *indigo* may also occur, due to a decomposition of indoxyl-glycuronic acid.

Urinary Calculi.

Besides certain pathological constituents of the urine, all those urinary constituents which occur as sediments take part in the formation of the urinary calculi. EBSTEIN[1] considers the essential difference between an amorphous or crystalline sediment in the urine on one side and urinary sand or large calculi on the other to be the occurrence of an organic frame in the last. As the sediments which appear in normal acid urine and in a urine alkaline through fermentation are different, so also are the urinary calculi which appear under corresponding conditions.

If the formation of a calculus and its further development take place in an undecomposed urine, it is called a PRIMARY formation. If, on the contrary, the urine has undergone alkaline fermentation and the ammonia formed thereby has given rise to a calculous formation by precipitating ammonium urate, triple phosphate, and earthy phosphates, then it is called a SECONDARY formation. Such a formation takes place, for instance, when a foreign body in the bladder produces catarrh accompanied by alkaline fermentation.

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[1] Die Natur und Behandlung der Harnsteine. Weisbaden, 1884.

We discriminate between the nucleus or nuclei—if such can be seen—and the different layers of the calculus. The nucleus may be essentially different in different cases, for quite frequently it consists of a foreign body introduced into the bladder. The calculus may have more than one nucleus. In a tabulation made by ULTZMANN of 545 cases of urinary calculi, the nucleus in 80.9% of the cases consisted of uric acid (and urates); in 5.6%, of calcium oxalate; in 8.6%, of earthy phosphates; in 1.4%, of cystin; and in 3.3%, of some foreign body.

During the growth of a calculus it often happens that, for some reason or other, the original calculus-forming substance is covered with another layer of a different substance. A new layer of the original substance may deposit on the outside of this, and this process may be repeated. In this way a calculus consisting originally of a simple stone may be converted into a so-called compound stone with several layers of different substances. Such calculi are always formed when a primary formation is changed into a secondary. By the continued action of an alkaline urine containing pus, the primary constituents of an originally primary calculus may be partly dissolved and be replaced by phosphates. Metamorphosed urinary calculi are formed in this way.

Uric-acid calculi are very abundant. They are variable in size and form. The size of the bladder-stone varies from that of a pea or bean to that of a goose-egg. Uric-acid stones are always colored; generally they are grayish yellow, yellowish brown, or pale red-brown. The upper surface is sometimes entirely even or smooth, sometimes rough or uneven. Next to the oxalate calculus, the uric-acid calculus is the hardest. The fractured surface shows regular concentric, unequally colored layers which may often be removed as shells. These calculi are formed primarily. Layers of uric acid sometimes alternate with other layers of primary formation, most frequently with layers of calcium oxalate. The simple uric-acid calculus leaves very little residue when burnt on platinum foil. It gives the murexid test, but there is no material development of ammonia when acted on by caustic soda.

Ammonium-urate calculi occur as primary calculi in new-born or nursing infants, rarely in grown persons. They often occur as a secondary formation. The primary stones are small, with a pale-yellow or dark-yellowish surface. When moist they are almost like dough; in the dry state they are earthy, easily crumbling into a pale powder. They give the murexid test, and develop much ammonia with caustic soda.

Calcium-oxalate calculi are, next to uric-acid calculi, the most abundant. They are either smooth and small (HEMP-SEED CALCULI) or larger, of the size of a hen's egg, with rough, uneven surface, or their surface is covered with prongs (MULBERRY CALCULI). These calculi produce bleeding easily, and therefore they often have a dark-brown surface due to decomposed blood-coloring matters. Among the calculi occurring in man these are the hardest. They dissolve in hydrochloric acid without developing gas, but are not soluble in acetic acid. After gently heating the powder it dissolves in acetic acid with frothing. After strongly heating the powder it is alkaline, due to the production of quicklime.

Phosphate Calculi. These, which consist mainly of a mixture of the normal phosphate of the alkaline earths with triple phosphate, may be very large. They are as a rule of secondary formation, and contain besides these phosphates also some ammonium urate and calcium oxalate. These calculi ordinarily consist of a mixture of these three constituents, earthy phosphate, triple phosphate, and ammonium urate, surrounding a foreign body as a nucleus. Their color is variable—white, dingy white, pale yellow, sometimes violet or lilac-colored (from indigo-red). The surface is always rough. Calculi consisting of triple phosphate alone are seldom found. They are ordinarily small, with granular or radiated crystalline fracture. Stones of simple-acid calcium phosphate are also seldom obtained. They are white and have a beautiful crystalline texture. The phosphatic calculi do not burn up, and the powder dissolves in acid without effervescence, and the solution gives the reactions for phosphoric acid and alkaline earths. The triple-phosphate calculi generate ammonia on the addition of an alkali.

Calcium-carbonate calculi occur chiefly in herbivora. They are seldom found in man. They have mostly chalky properties, and are ordinarily white. They are completely or in great part dissolved by acids with effervescence.

Cystin calculi occur but seldom. They are of primary formation, of various sizes, sometimes attaining the size of a hen's egg. They have a smooth or rough surface, are white or pale yellow, and have a crystalline fracture. They are not very hard; they burn up almost entirely on platinum foil, burning with a bluish flame. They give the above-mentioned reactions for cystin.

Xanthin calculi are very rarely found. They are also of primary formation. They vary from the size of a pea to that of a hen's egg. They are whitish, yellowish brown or cinnamon-brown in color, medium hard, with amorphous fracture, and on rubbing appear like wax. They burn up completely when heated on platinum foil. They give the xanthin reaction with nitric acid and alkali, but this must not be mistaken for the murexid test.

Urostealith calculi have only been observed a few times. In the moist state they are soft and elastic at the temperature of the body, but in the dry state they are brittle, with an amorphous fracture and waxy appearance. They burn with an illuminating flame when heated on platinum foil, and generate an odor similar to resin or shellac. Such a calculus, investigated by KRUKENBERG,[1] consisted of paraffine derived from a paraffine bougie used as a sound on the patient. Perhaps the urostealith calculi observed in other cases had a similar origin, although the substances of which they consisted have not been closely studied. HORBACZEWSKI has recently analyzed a case of urostealith which, to all appearances, was formed in the bladder. This calculus contained 25 p. m. water, 8 p. m. inorganic bodies, 117 p. m. bodies insoluble in ether, and 850 p. m. organic bodies soluble in ether, among which were 515 p. m. free fatty acids, 335 p. m. fat, and traces of chloresterin. The fatty acids consisted of a mixture of stearic, palmitic, and probably myristic acids.

HORBACZEWSKI[2] has also analyzed a bladder-stone which contained 958.7 p. m. *cholesterin*.

Fibrin calculi sometimes occur. They consist of more or less changed fibrin coagulum. On burning they develop an odor of burnt horn.

The *chemical investigation of urinary calculi* is of great practical importance. To make such an examination actually instructive it is necessary to investigate separately the different layers which constitute the calculus. For this purpose saw the calculus, which has been wrapped in paper, with a fine saw so that the nucleus is sawed through and accessible. Then peel off the different layers, or, if the stone is to be kept, scrape off enough of the powder from each layer for examination. This powder is then tested by heating on platinum foil. It must not be forgotten that a calculus is never entirely burnt up, and also that it is never so free from organic matter that on heating it does not carbonize. Do not, therefore, lay too great stress on a very insignificant unburnt residue or on a very small amount of organic matter, but consider the calculus in the former case as completely burnt and in the latter as not burnt.

When the powder is in great part burnt up, but a significant quantity of unburnt residue remains, then the powder in question contains as a rule urates mixed with inorganic bodies. In such cases remove the urate with boiling water, and then test the filtrate for uric acid and the expected bases. The residue is then tested according to the following *schema* of HELLER, which is well adapted to the investigation of urinary calculi. In regard to more detailed examination the reader is referred to special works on the subject.

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[1] Chem. Untersuch. z. wissensch. Med., Bd. 2. Cited from Maly's Jahresber., Bd. 19, S. 422.

[2] Zeitschr. f. physiol. Chem., Bd. 18.

On heating the powder on platinum foil it

Does not burn	The powder when treated with HCl	Does not effervesce	The gently heated powder with HCl	The powder when moistened with a little KHO	Abundant ammonia. The powder dissolves in acetic acid or HCl. This solution gives a crystalline precipitate with ammonia	Triple phosphate (mixed with unknown amount of earthy phosphate).
					No NH_3, or at least only traces of NH_3. Powder dissolves in acetic acid or HCl. This solution is precipitated by ammonia (amorphous)	Bone-earth (phosphate of calcium and magnesium).
				Effervesces		Calcium oxalate.
		Effervesces				Calcium carbonate.
Does burn	With flame				Flame yellow, continuous. Odor of burnt feathers. Insoluble in alcohol and ether. Soluble in KHO with heat. Precipitated herefrom by acetic acid and generation of H_2S	Fibrin.
					Flame yellow, pale, continuous. Odor of resin or shellac on burning. Powder soluble in alcohol and ether	Urostealith.
					Flame pale blue, burns a short time. Peculiar sharp odor. The powder dissolves in ammonia, and six-sided plates separate on the spontaneous evaporation of the ammonia	Cystin.
	Without flame				**Does not give the murexid test. The powder dissolves in HNO_3 without effervescence. The dried yellow residue becomes orange with alkali, beautiful red on warming**	**Xanthin.**
		The powder gives the murexid test	The powder when treated with KHO gives		Strong ammonia reaction	Ammonium urate.
					No noticeable ammonia reaction	Uric acid.

CHAPTER XVI.

THE SKIN AND ITS SECRETIONS.

IN the structure of the skin of man and vertebrates many different kinds of substances occur which have already been treated of, such as the constituents of the epidermis formation, the connective and fatty tissues, the nerves, muscles, etc. Among these the different horn-formations, the hair, nails, etc., whose chief constituent, keratin, has been spoken of in another chapter (Chap. II), are of special interest.

The cells of the horny formation show, in proportion to their age, a different resistance to chemical reagents, especially fixed alkalies. The younger the horn-cell the less resistance it has to the action of alkalies; with advancing age the resistance becomes greater, and the cell-membranes of many horn-formations are nearly insoluble in caustic alkalies. Keratin occurs in the horn-formation mixed with other bodies, from which it is isolated with difficulty. Among these bodies the mineral constituents in many cases occupy a prominent place because of their quantity. Hair leaves on burning 5-70 p. m. ash, which may contain in 1000 parts 230 parts alkali sulphates, 140 parts calcium sulphate, 100 parts iron oxide, and even 400 parts silicic acid. Dark hair on burning seems generally, but not always, to yield more iron oxide than blond. The nails are rich in calcium phosphate, and the feathers rich in silicic acid.

The granules occurring in the stratum granulosum of the skin consist of a substance which has been called *eleidin*, and which is considered as an intermediate step in the transformation of the protoplasm into keratin. The chemical nature of this substance is unknown.

The skin of invertebrates has been the subject, in a few cases, of chemical investigation, and in these animals various substances have been found, of which a few, though little studied, are worth discussing. Among these bodies *tunicin*, which is found especially in the tunic of the tunicata, and the widely diffused *chitin*, found in the cuticle-formation of invertebrates, are of interest.

Tunicin. Cellulose seems, according to the investigations of AMBRONN,[1] to occur rather extensively in the animal kingdom in the arthropoda and the mollusks. It has been known for a long time as the tunic of the *tunicata*, and this animal cellulose was called tunicin by BERTHELOT.[2] According to the recent investigations of WINTERSTEIN[3] there does not seem to exist any marked difference between tunicin and ordinary cellulose. On boiling with dilute acid tunicin yields dextrose, as shown first by FRANCHIMONT[4] and later confirmed by WINTERSTEIN.

Chitin is not found in vertebrates. In invertebrates chitin is alleged to occur in several classes of animals; but it can only be positively asserted that true, typical chitin is found only in articulated animals, in which it forms the chief organic constituent of the shell, etc. According to KRAWKOW[5] chitin of the shell, etc., does not seem to occur free, but in combination with another substance, probably a proteid-like body.

According to SUNDWIK[6] the composition of chitin is probably $C_{60}H_{100}N_8O_{38} + n(H_2O)$, where n may vary between 1 and 4, and it is probably an amine derivative of a carbohydrate, with the general formula $n(C_{12}H_{20}O_{10})$. According to KRAWKOW[7] chitin shows different origins by its unequal behavior with iodine, and he therefore concludes that there must exist quite a group of chitins, which seem to be amine derivatives of different carbohydrates such as dextrose, glycogen, dextrins, etc. Chitin is decomposed on boiling with mineral acids and yields, as shown by LEDDERHOSE,[8] *glucosamine* and *acetic acid*. SCHMIEDEBERG[9] therefore considers chitin as a probable acetyl acetic acid combination of glucosamine. If, as previously mentioned (page 345), the chondroitic-sulphuric acid contains a glucosamine group, as made probable by the investigations of SCHMIEDEBERG, then, according to SCHMIEDEBERG, glucosamine forms the bridge which leads from the chitin of lower animals to the cartilage of higher organized beings. According to the recent investigations of GILSON[1] and WINTERSTEIN[2] several fungi seem to contain chitin instead of cellulose. On heating chitin with alkali and a little water to 180° C. a cleavage takes place, according to HOPPE-SEYLER and ARAKI,[3] with the formation of a new substance, *chitosan*, $C_{14}H_{26}N_2O_{10}$, which retains the shape of the original chitin and the splitting off of acetic acid. Chitosan is dissolved by dilute acids, also acetic acid, and is colored violet by a dilute iodine solution. It splits into acetic acid and glucosamine by the action of hydrochloric acid. On heating with acetic anhydride it is converted into a chitin-like substance, which is not identical with chitin and contains at least three acetyl groups.

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[1] Maly's Jahresber., Bd. 20, S. 318.

[2] Annal. de chim. et phys., Tome 56, Compt. rend., Tome 47.

[3] Zeitschr. f. physiol. Chem., Bd. 18.

[4] Ber. de deutsch. chem. Gesellsch., Bd. 12.

[5] Zeitschr. f. Biologie, Bd. 29.

[6] Zeitschr. f. physiol. Chem., Bd. 5.

[7] L. c.

[8] Zeitschr. f. physiol. Chem., Bdd. 2 u. 4.

[9] Arch. f. exp. Path. u. Pharm., Bd. 28.

In the dry state chitin forms a white, brittle mass retaining the form of the original tissue. It is insoluble in boiling water, alcohol, ether, acetic acid, dilute mineral acids, and dilute alkalies. It is soluble in concentrated acids. It is dissolved without decomposing in cold concentrated hydrochloric acid, but is decomposed by boiling hydrochloric acid. When chitin is dissolved in concentrated sulphuric acid and the solution dropped into boiling water and then boiled, we obtain a substance (glucosamine or glucose) which reduces copper suboxide in alkaline solutions. According to KRAWKOW the various chitins behave differently with iodine or with sulphuric acid and iodine in that some are colored reddish brown, blue, or violet, while others are not colored at all.

Chitin may be easily prepared from the wings of insects or from the shells of the lobster or the crab, the last mentioned having first been extracted by an acid so as to remove the lime-salts. The wings or shells are boiled with caustic alkali until they are white, afterward washed with water, then with dilute acid and water, and lastly extracted with alcohol and ether. If chitin so prepared is dissolved in cold, concentrated sulphuric acid and diluted with cold water, then pure chitin separates out, having been set free from the combination with the other body (KRAWKOW).

Hyalin is the chief organic constituent of the walls of hydatid cysts. From a chemical point of view it stands close to chitin, or between it and the proteid. In old and more transparent sacs it is tolerably free from mineral bodies, but in younger sacs it contains a great quantity (16%) of lime-salts (carbonate, phosphate, and sulphate).

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[1] Compt. rend., Tome 120.

[2] Ber. d. deutsch. chem. Gesellsch., 1894–1895.

[3] Zeitschr. f. physiol. Chem., Bd. 20.

According to LÜCKE[1] its composition is:

	C	H	N	O
From old cysts	45.3	6.5	5.2	43.0
From young cysts	44.1	6.7	4.5	44.7

It differs from keratin on the one hand and from proteids on the other by the absence of sulphur, also by its yielding, when boiled with dilute sulphuric acid, a variety of sugar in large quantities (50%), which is reducing, fermentable, and dextrogyrate. It differs from chitin by the property of being gradually dissolved by caustic potash or soda, or by dilute acids; also by its solubility on heating with water to 150° C.

The coloring matters of the skin and horn-formations are of different kinds, but have not been much studied. Those occurring in the Malpighian layer of the skin, especially of the negro, and the black or brown pigment occurring in the hair belong to the group of coloring matters which have received the name *melanins*.

Melanins. This group includes several different varieties of amorphous black or brown pigments which are insoluble in water, alcohol, ether, chloroform, and dilute acids, and which occur in the skin, hair, epithelium-cells of the retina, in sepia, in certain pathological formations, and in the blood and urine in disease. Of these pigments there are a few, such as the melanin of the eye and that from the melanotic sarcomata of horses, the *hippomelanin* (NENCKI and BERDEZ[2]), which are soluble with difficulty in alkalies, while others, such as the pigment of the hair and the coloring matter of certain pathological swellings in man, the *phymatorusin* (NENCKI and BERDEZ), are easily soluble in alkalies.

Among the melanins there are a few, for example, the choroid pigment, which are free from sulphur; others, on the contrary, as the pigment of the hair and of horse-hair, are rather rich in sulphur (2–4%), while the phymatorusin found in certain swellings and in the urine (NENCKI and BERDEZ, K. MÖRNER[3]) is very rich in sulphur (8–10%). Whether any of these pigments, especially the phymatorusin, contains any iron or not is an important though disputed point, for it leads to the question whether these pigments are formed from the blood-coloring matters. The pigment, phymatorusin, isolated by NENCKI and BERDEZ from melanotic sarcomata, is, according to them, free from iron and is not a derivative of hæmoglobin. K. MÖRNER and later also BRANDL and L. PFEIFFER[4] found, on the contrary, that this pigment did contain iron, and they consider it as a derivative of the blood-pigments. The difficulties which attend the isolation and purification of the melanins have not been overcome in certain cases, while in others it is questionable whether the final product obtained has not another composition than the original coloring matter, owing to the energetic chemical processes resorted to in its purification. Under such circumstances it seems that a tabulation of the analyses of different melanin preparations made up to the present time are of secondary importance.

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[1] Virchow's Arch., Bd. 19.

[2] Arch. f. exp. Path. u. Pharm., Bdd. 20 u. 24.

[3] Zeitschr. f. physiol. Chem., Bd. 11, which contains all the older literature, and Bd. 12.

[4] Zeitschr. f. Biologie, Bd. 26.

Among the above-mentioned bodies belonging to the melanin group, the phymatorusin prepared by NENCKI and SIEBER from melanotic sarcomata, and that prepared by K. MÖRNER from the sarcomata and the urine of a patient, seem to be of special interest. Phymatorusin is an amorphous dark-brown pigment soluble in alkalies or alkali carbonates, but insoluble in warm 50–75% acetic acid. In alkaline solution it shows no absorption-bands. According to NENCKI and SIEBER it is free from iron, but MÖRNER, on the contrary, claims that it does contain iron. MÖRNER found for this coloring matter from tumors (A) and from urine (B) the following composition calculated on the substance considered as ash-free:

	A	B
C	55.32—56.13	55.76
H	5.65— 6.33	5.95
N	12.30	12.27
S	7.97	9.01
Fe	0.063—0.081	0.20

NENCKI and SIEBER have also shown that other melanins, not identical with phymatorusin, occur in melanotic sarcomata of man. The investigations of BRANDL and PFEIFFER seem to lead to a similar conclusion.

The coloring matter or matters of human hair contain a low quantity of nitrogen, 8.5% (SIEBER[1]), and a variable but high quantity of sulphur, 2.71–4.10%. The considerable quantity of iron oxide found in the ash does not seem to belong to the pigments.

In addition to the coloring matters of the human skin we may also here treat of the pigments found in the skin or epidermis-formation of animals.

The beautiful color of the feathers of many birds depends in certain cases on purely physical causes (interference-phenomena), but in other cases on coloring matters of various kinds. Such a coloring matter is the amorphous reddish violet *turacin*, which contains 7% copper, and whose spectrum is very similar to that of oxyhæmoglobin. KRUKENBERG[2] found a large number of coloring

[1] Arch. f. exp. Path. u. Pharm., Bd. 20.

[2] See Physiol. Studien, Abth. 5, u. 2, Reih. Abth. 1, S. 151, Abth. 2, S. 1, und Abth. 3, S. 128.

matters in birds' feathers, namely, *zooerythrin*, *zoofulvin*, *turacoverdin*, *zoorubin*, *psittacofulvin*, and others which cannot be enumerated here.

Tetronerythrin, so named by WURM,[1] is a red amorphous pigment, which is soluble in alcohol and ether, and which occurs in the red warty spots over the eyes of the heath-cock and the grouse, and which is very widely spread among the invertebrates (HALLIBURTON,[2] DE MEREJKOWSKI,[3] MACMUNN[4]). Besides tetronerythrin MACMUNN found in the shells of crabs and lobsters a blue coloring matter, *cyanocrystallin*, which turns red with acids and by boiling water. *Hæmatoporphyrin*, according to MACMUNN,[5] also occurs in the integuments of certain lower animals.

In addition to the coloring matters thus far mentioned a few others found in certain animals (though not in the skin) will be spoken of.

Carminic acid, or the red coloring matter of cochineal, has the composition $C_{17}H_{18}O_{10}$. It gives sugar on boiling with acids, but this does not correspond with the recent statements of LIEBERMANN.[6] The beautiful purple solution of ammonium carminate has two absorption-bands between *D* and *E* which are similar to those of oxyhæmoglobin. These bands lie nearer to *E* and closer together and are less sharply defined. *Purple* is the evaporated residue from the purple-violet secretion, caused by the action of the sunlight, from the so-called "purple gland" of the tunic of certain species of *murex* and *purpura*. Its chemical nature has not been investigated.

Among the remaining coloring matters found in invertebrates we may mention *blue stentorin*, *actiniochrom*, *bonellin*, *polyperythrin*, *pentacrinin*, *antedonin*, *crustaceorubin*, *janthinin*, and *chlorophyll*.

Sebum when freshly secreted is an oily semi-fluid mass which solidifies on the upper surface of the skin, forming a greasy coating. The quantity is very different in different persons. HOPPE-SEYLER[7] has found a body similar to casein, besides albumin and fat, in the sebum. Cholesterin is also found in this fat, and in especially large quantities in the *vernix caseosa*. The solids of the sebum consist chiefly of fat, epithelium-cells, and protein bodies; the *vernix caseosa* consists chiefly of fat.

On account of the generally diffused view that wax of the plant epidermis serves as protection for the inner parts of the fruit and plant, LIEBREICH[8] has suggested that the combinations of fatty acids with monatomic alcohols are the reason for the resistance property of the waxes as compared with the glycerin fats. He also considers that the cholesterin fats play the rôle of a protective fat in the animal kingdom, and he has been able to detect cholesterin fat in human skin and hair, in vernix caseosa, whale-bone, tortoise-shell, cow's horn, the feathers and beaks of several birds, the prickles of the hedgehog and porcupine, the hoofs of horses, etc. He draws the following conclusion from this, namely, that the cholesterin fats always appear in combination with the keratinous substance, and that the cholesterin fat, like the wax of plants, serves as protection for the skin-surface of animals.

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[1] Zeitschr. f. wissensch. Zool., 1871. Cited from Maly's Jahresber., Bd. I, S. 52.

[2] Journal of Physiol., Vol. 6.

[3] Compt. rend., Tome 93.

[4] Proc. Roy. Soc., 1883.

[5] Quart. Journ. of Micros. Sc., 1877, and Journal of Physiol., Vol. 7.

[6] Ber. d. deutsch. chem. Gesellsch., Bd. 18.

[7] Physiol. Chem., S. 760.

[8] Virchow's Arch., Bd. 121.

Cerumen is a mixture of the secretion of the sebaceous and sweat glands of the cartilaginous part of the outer organs of hearing. It contains chiefly soaps and fat, and besides these a red substance easily soluble in alcohol and with a bitter-sweet taste.

The **preputial secretion**, *smegma præputii*, contains chiefly fat, also cholesterin and ammonium soaps, which probably are produced from decomposed urine. The hippuric acid, benzoic acid, and calcium oxalate found in the smegma of the horse have probably the same origin.

We may also consider as a preputial secretion the *castoreum*, which is secreted by two peculiar glandular sacs in the prepuce of the beaver. This castoreum is a mixture of proteids, fat, resins, traces of phenol (volatile oil), and a non-nitrogenized body, *castorin*, crystallizing in four-sided needles from alcohol, insoluble in cold water, but somewhat soluble in boiling water, and whose composition is little known.

Wool-fat, or the so-called fat-sweat of sheep, is a mixture of the secretion of the sudoriparous and sebaceous glands. We find in the watery extract a large quantity of potassium which is combined with organic acid, volatile and non-volatile fatty acids, benzoic acid, phenol-sulphuric acid, lactic acid, malic acid, succinic acid, and others. The fat contains among other bodies abundant quantities of ethers of fatty acids with cholesterin and isocholesterin.

The secretion of the coccygeal glands of ducks and geese contains a body similar to casein, besides albumin, nuclein, lecithin, and fat, but no sugar (DE JONGE[1]). Poisonous bodies have been found in the secretion of the skin of the salamander and the toad respectively, *samandarin* (ZALESKY[2]) and *bufidin* (JORNARA and CASALI[3]).

The Sweat. Of the secretions of the skin, whose quantity amounts to about $\frac{1}{64}$ of the weight of the body, a disproportionally large part consists of water. Next to the kidneys, the skin in man is the most important means for the elimination of water. As the glands of the skin and the kidneys stand near to each other in regard to their functions, they may to a certain extent act vicariously for one another.

The circumstances which influence the secretion of sweat are very numerous, and the quantity of sweat secreted must consequently vary very considerably. The secretion differs for different parts of the skin, and it has been stated that the perspiration of the cheek, that of the palm of the hand, and that under the arm stand to each other as 100 : 90 : 45. From the unequal secretion on different parts of the body it follows that no results as to the quantity of secretion for the entire surface of the body can be calculated from the quantity secreted by a small part of the skin in a given time. In determining the total quantity a stronger secretion is as a rule produced, and as the glands can with difficulty work for a long time with the same energy, it is hardly correct to estimate the quantity of secretion per 24 hours from a strong secretion enduring only a short time.

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[1] Zeitschr. f. physiol Chem., Bd. 3.

[2] Hoppe-Seyler's Med chem. Untersuch., S. 85.

[3] Riv. di Bologna, 1873. Cited from Maly's Jahresber., Bd. 3, S. 64.

The perspiration obtained for investigation is never quite pure, but contains cast-off epidermis-cells, also cells and fat-globules from the sebaceous glands. Filtered sweat is a clear, colorless fluid with a salty taste and of different odors from different parts of the body. The physiological reaction is acid, according to most statements. Under certain conditions also an alkaline sweat may be secreted (TRÜMPY and LUCHSINGER,[1] HEUSS[2]). An alkaline reaction may also depend on a decomposition with the formation of ammonia. According to a few investigators the physiological reaction is alkaline, and an acid reaction depends, according to these investigators, upon an admixture of fatty acids from the sebum. MORIGGIA[3] found that the sweat from herbivora was ordinarily alkaline, while that from carnivora was generally acid. According to SMITH[4] horse's sweat is strongly alkaline. The specific gravity of human sweat is 1.003–1.005.

Perspiration contains 977.4–995.6 p. m., average 988.2 p. m., water, and 4.4–22.6 p. m., average 11.80 p. m., solids. The organic bodies are *neutral fats*, *cholesterin*, *volatile fatty acids*, traces of proteid (according to LECLERC[5] and SMITH[6] habitually in horses, according to GAUBE[7] regularly in man, and according to

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[1] Pflüger's Arch., Bd. 18.

[2] Monatshefte f. prakt. Dermat., Bd. 14. Cited from Maly's Jahresber., Bd. 22, S. 193.

[3] Moleschott, Untersuch. zur Naturhre, Bd. 11; also Maly's Jahresber., Bd. 3, S. 126.

[4] Journal of Physiol., Bd. 11. In regard to the older literature on sweat see Hermann's Handbuch, Bd. 5, Thl. 1, S. 421 u. 543.

[5] Compt. rend., Tome 107.

[6] L. c.

[8] Maly's Jahresber., Bd. 22, S. 193.

LEUBE[1] sometimes after hot baths, in BRIGHT's disease, and after the use of pilocarpin), also *creatinin* (CAPRANICA[2]), *aromatic oxyacids*, *ethereal-sulphuric acids* of *phenol* and *skatoxyl* (KAST[3]), but not of indoxyl, and lastly *urea.* The quantity of urea has been determined by ARGUTINSKY.[4] In two steam-bath experiments, in which in the course of $\frac{1}{2}$ and $\frac{3}{4}$ hour respectively he obtained 225 and 330 c. c. sweat, he found 1.61 and 1.24 p. m. urea. Of the total nitrogen of the sweat in these two experiments 68.5 and 74.9% respectively belong to the urea. From ARGUTINSKY's experiments, and also from those of CRAMER,[5] it follows that of the total nitrogen a portion not to be disregarded is eliminated by the sweat. This portion was indeed 12% in an experiment of CRAMER at high temperature and powerful muscular activity. CRAMER has also found ammonia in the sweat. In uræmia, and in ischuria in cholera, urea may be secreted in such quantities by the sweat-glands that crystals deposit upon the skin. The mineral bodies consist chiefly of sodium chloride with some potassium chloride, alkali sulphate, and phosphate. The relative quantities of these in perspiration differ materially from the quantities in the urine (FAVRE,[6] KAST). The relationship, according to KAST, is as follows:

	Chlorine	:	Phosphate	:	Sulphate
In perspiration	1	:	0.0015	:	0.009
In urine	1	:	0.1320	:	0.397

KAST found that the proportion of ethereal-sulphuric acid to the sulphate sulphuric acid in sweat was 1 : 12. After the administration of aromatic substances the ethereal-sulphuric acid does not increase to the same extent in the sweat as in the urine (see Chapter XV).

Sugar may pass into the sweat in diabetes, but the passage of the bile-coloring matters has not been positively shown in this secretion. *Benzoic acid*, *succinic acid*, *tartaric acid*, *iodine*, *arsenic*, *mercuric chloride*, and *quinine* pass into the sweat. *Uric acid* has also been found in the sweat in gout, and *cystin* in cystinura.

Chromhidrosis is the name given to the secretion of colored sweat. Sometimes sweat has been observed to be colored blue by indigo (BIZIO[7]), by pyocyanin, or by ferro-phosphate (KOLLMANN[1]). True blood-sweat, in which blood-corpuscles exude from the openings of the glands, have also been observed.

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[1] Leube, Virchow's Arch., Bdd. 48 u. 50, and Arch. f. klin. Med., Bd. 7.

[2] Maly's Jahresber., Bd. 12, S. 190.

[3] Zeitschr. f. physiol. Chem., Bd. 11, S. 501.

[4] Pflüger's Arch., Bd. 46.

[5] Arch. f. Hygiene, Bd. 10.

[6] Compt. rend., Tome 35, and Arch. génér. de méd., 1853 (Sér. 5), Vol. 2.

[7] Wien. Sitzungsber., Bd. 39.

The *exchange of gas through the skin* in man is of very little importance compared with the exchange of gas by the lungs. The absorption of oxygen by the skin, which was first shown by REGNAULT and REISET, is very small. The quantity of carbon dioxide eliminated by the skin increases with the rise of temperature (AUBERT,[2] RÖHRIG,[3] FUBINI and RONCHI[4]). It is also greater in light than in darkness. It is greater during digestion than when fasting, and greater after a vegetable than after an animal diet (FUBINI and RONCHI). The quantity calculated by various investigators for the entire skin surface in 24 hours varies between 2.23 and 32.8 grms.[5] In certain animals, as in frogs, the exchange of gas through the skin is of great importance.

As the exchange of gas through the skin in man and mammalia is very small, it follows that the injurious and dangerous effects caused by covering the skin with varnish, oil, or the like can hardly depend on a prevented exchange of gas. After varnishing the skin there is a considerable loss of heat, and the animal quickly dies. If the animal, on the contrary, be guarded from this loss of heat, it may be saved, or at least kept alive for a longer time. This effect was supposed to be due to a poisoning caused by a retention of one or more substances of the perspiration (*perspirabile retentum*), accompanied by fever and increased loss of heat through the skin; but this statement has not been substantiated. This phenomenon seems to be due to other causes, and at least in certain animals (rabbits) death seems to ensue from the paralysis of the vaso-motor nerves. In anastomosis the loss of heat through the skin seems to be increased to such an extent that the animal dies from the lowered temperature.

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[1] Wurzb. med. Zeitsch., Bd. 7, S. 251. Cited from Gorup-Besanez, Lehrb., 4. Aufl., S. 555.

[2] Pflüger's Arch., Bd. 6.

[3] Deutsch. klin., 1872, S. 209.

[4] Moleschott's Metersuch. zur Naturhhre., Bd. 12.

[5] See Hoppe-Seyler, Physiol. Chem. S. 580.

CHAPTER XVII.

CHEMISTRY OF RESPIRATION.

During life a constant exchange of gases takes place between the animal body and the surrounding medium. Oxygen is inspired and carbon dioxide expired. This exchange of gases, which is called respiration, is brought about in man and vertebrates by the nutritive fluids, blood and lymph, which circulate in the body and which are in constant communication with the outer medium on one side and the tissue-elements on the other. Such an exchange of gaseous constituents may take place wherever the anatomical conditions offer no obstacle, and in man it may go on in the intestinal tract, through the skin, and in the lungs. As compared with the exchange of gas in the lungs, the exchange already mentioned which occurs in the intestine and through the skin is very insignificant. For this reason we will discuss in this chapter only the exchange of gas between the blood and the air of the lungs on one side, and the blood and lymph and the tissues on the other. The first is often designated external respiration, and the other internal respiration.

In this chapter we will accordingly first discuss the gases of the blood and lymph, and then the exchange of gas in the lungs and tissues. The quantitative circumstances of the exchange of gas stand in such close relationship to metabolism in general that they will be treated of in the last chapter, on the income and output of the body under different conditions. Only the chief points in the methods commonly employed for measuring the exchange of gas will be mentioned.

I. The Gases of the Blood.

Since the pioneer investigations of Magnus and Lothar Meyer the gases of the blood have formed the subject of repeated, careful investigations by prominent experimenters, among whom we must mention first C. Ludwig and his pupils and E. Pflüger and his school. By these investigations not only has science been enriched by a mass of facts, but also the methods themselves have been made more perfect and accurate. In regard to these methods, as also in regard to the laws of the absorption of gases by liquids, dissociation, and other questions belonging here, the reader is referred to complete text-books on physiology, on physics, and on gasometric analysis.

The gases occurring in blood under physiological conditions are *oxygen*, *carbon dioxide*, and *nitrogen*. The last-mentioned gas is found only in very small quantities, on an average of 1.8 vol. per cent. The quantity is here, as in all following experiments, calculated for 0° C. and 760 mm. pressure. The nitrogen seems to be simply absorbed into the blood, at least in great part. It appears to play no part in the processes of life, and its quantity varies but slightly in the blood of different blood-vessels.

The oxygen and carbon dioxide behave otherwise, as their quantities have significant variations, not only in the blood from different blood-vessels, but also because many conditions, such as a difference in the rapidity of circulation, a different temperature, rest and activity, cause a change. In regard to the gases they contain the greatest difference is observable between the blood of the arteries and that of the veins.

The *quantity of oxygen* in the arterial blood of dogs is on an average 22 vols. per cent (PFLÜGER). In human blood SETSCHENOW found about the same quantity, namely, 21.6 vols. per cent. Lower figures have been found for rabbit's and bird's blood, respectively 13.2% and 10–15% (WALTER, JOLYET). Venous blood has very variable quantities of oxygen. LUDWIG and SCZELKOW found 6.8% oxygen in the venous blood of resting muscles, and a still smaller quantity in the venous blood of active muscles. Oxygen is entirely absent from blood after asphyxiation, or occurs only as traces. The venous blood of the glands seems, on the contrary, during secretion to be richer in oxygen than ordinary venous blood. By summarizing a great number of analyses by different experimenters ZUNTZ has calculated that the venous blood of the right side of the heart contains on an average 7.15% less oxygen than the arterial blood.

The *quantity of carbon dioxide* in the arterial blood of dogs is 30 to 40 vols. per cent (LUDWIG, SETSCHENOW, PFLÜGER, P. BERT, and others), most generally about 40%. SETSCHENOW found 40.3 vols. per cent in human arterial blood. The quantity of carbon dioxide in venous blood varies still more (LUDWIG, PFLÜGER and their pupils, P. BERT, MATHIEU and URBAIN, and others). According to the calculations of ZUNTZ the venous blood of the right side of the heart contains about 8.2% more carbon dioxide than the arterial. The average amount may be put down as 48 vols. per cent. HOLMGREN found in blood after asphyxiation even 69.21 vols. per cent carbon dioxide.[1]

Oxygen is absorbed only to a small extent by the plasma or serum, in which PFLÜGER found but 0.26%. The greater part or nearly all of the oxygen is loosely combined with the hæmoglobin. The quantity of oxygen which is contained in the blood of the dog corresponds closely to the quantity which from the activity of the hæmoglobin we should expect to combine with oxygen, and also the quantity of hæmoglobin in canine blood. It is difficult to ascertain how far the circulating arterial blood is saturated with oxygen, as immediately after bleeding a loss of oxygen always takes place. Still it seems to be unquestionable that it is not quite completely saturated with oxygen in life.

The question whether ozone occurs in the blood is to be answered decidedly in the negative. It is not only impossible to detect ozone in the blood, but the possibility of the occurrence of ozone in the fluids and tissues is even *a priori* to be denied. Ozone acts as nascent oxygen; and as easily oxidizable substances occur in the organism which combine with nascent oxygen, ozone, if such a formation should take place at all, would be destroyed instantly. But such a formation of ozone in the animal body cannot be admitted. Ozone may indeed be formed by slow oxidation, since the nascent oxygen formed in consequence combines with neutral oxygen, forming ozone; but in the animal organism the nascent oxygen must be combined with the oxidizable substances before it can form ozone.

It was formerly believed that the hæmoglobin acted as an "ozone-exciter," possessing the property of converting the inactive oxygen of the air into ozone. The red blood-corpuscles can by themselves also give a blue color with tincture of guaiacum, which is markedly seen when this tincture is dried on blotting-paper and a drop of blood previously diluted with 5–10 vols. water is added.

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[1] All the figures given above may be found in Zuntz's "Die Gase des Blutes" in Hermann's Handbuch d. Physiol., Bd. 4, Thl. 2, S. 33–43, which also contains detailed statements and the pertinent literature.

According to PFLÜGER,[1] we are here dealing (see page 134) with a decomposition and gradual oxidation of hæmoglobin, in which processes the neutral oxygen is split, setting free oxygen atoms.

The carbon dioxide of the blood occurs in part, and indeed, according to the investigations of ALEX. SCHMIDT,[2] ZUNTZ,[3] and L. FREDERICQ,[4] to the extent of at least one third, in the blood-corpuscles, and also in part, and in fact the greatest part, in the plasma and serum respectively.

The carbon dioxide of the red corpuscles is loosely combined, and the constituent uniting with the CO_2 of the same seems to be the alkali combined with phosphoric acid, oxyhæmoglobin or hæmoglobin and globulin on one side and the hæmoglobin itself on the other. That in the red corpuscles alkali phosphate occurs in such quantities that it may be of importance in the combination with carbon dioxide is not to be doubted, and we must admit that from the diphosphate, by a greater partial pressure of the carbon dioxide, monophosphate and alkali carbonate are formed, while by a lower partial pressure of the carbon dioxide the mass action of the phosphoric acid comes again into play, so that, with the carbon dioxide becoming free, a re-formation of alkali diphosphate takes place. It is generally admitted that the blood-coloring matters, especially the oxyhæmoglobin, which can expel carbon dioxide from sodium carbonates *in vacuo*, act like an acid; and as the globulins also act like acids (see below), this body may also occur in the blood-corpuscles as an alkali combination. The alkali of the blood-corpuscles must therefore, according to the law of mass action, be divided between the carbon dioxide, phosphoric acid, and the other constituents of the blood-corpuscles which are considered as acid acting, and among these especially the blood-pigments, as the globulin can hardly be of importance because of its small quantity. By greater mass action or greater partial pressure of the carbon dioxide, bicarbonate must be formed at the expense of the diphosphates and the other alkali combinations, while at a diminished partial pressure of the same gas, with the escape of carbon dioxide, the alkali diphosphate and the other alkali combinations must be re-formed at the cost of the bicarbonate.

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[1] Pflüger's Arch., Bd. 10, S. 252.

[2] Ber. d. k. sächs. Gesellsch. d. Wissensch., Math.-phys. Klasse, Bd. 19, 1867.

[3] Centralbl. f. d. med. Wissensch., 1867, S. 529.

[4] Recherches sur la constitution du Plasma sanguin, 1878, p. 50-51.

Hæmoglobin must nevertheless, as the investigations of SETSCHENOW[1] and ZUNTZ,[2] and especially those of BOHR[3] and TORUP,[4] have shown, be able to hold the carbon dioxide loosely combined even in the absence of alkali. BOHR has also found that the dissociation curve of the carbon-dioxide hæmoglobin corresponds essentially to the curve of the absorption of carbon dioxide, on which ground he and TORUP consider the hæmoglobin itself as of importance in the binding of the carbon dioxide of the blood and not its alkali combinations. In regard to this question the conditions are not quite clear. If carbon dioxide is allowed to act on hæmoglobin, it unites (BOHR, TORUP) with the colored atomic group of the hæmoglobin, splitting off proteid, and from this hæmoglobin, so decomposed, oxyhæmoglobin cannot be formed by the action of oxygen. According to BOHR, for each gramme of hæmoglobin at + 18.4° C. and a pressure of 30 mm. 2.4 c. cm. carbon dioxide are combined; and since in the arterial blood nearly all the hæmoglobin exists as oxyhæmoglobin, it is difficult to understand how the hæmoglobin can be of any great importance in the binding of the carbon dioxide of the blood. According to the recent investigations of BOHR[5] this condition is explained by the property of the hæmoglobin to take up both gases, carbon dioxide and oxygen, simultaneously and independently of each other. It takes place, as admitted by BOHR, by the oxygen probably uniting with the pigment nucleus, and the carbon dioxide with the proteid component.

The chief part of the carbon dioxide of the blood is found in the blood-plasma or the blood-serum, which follows from the fact that the serum is richer in carbon dioxide than the corresponding blood itself. By experiments with the air-pump on blood-serum it has been found that the chief part of the carbon dioxide contained in the serum is given off in a vacuum, while a smaller part can be pumped out only after the addition of an acid. The red corpuscles also act as an acid, and therefore in blood all the carbon dioxide is expelled *in vacuo*. Hence a part of the carbon dioxide is firmly chemically combined in the serum.

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[1] Centralbl. f. d. med. Wissensch., 1877. See also Zuntz in Hermann's Handbuch, S.76.

[2] L. c., S. 76.

[3] See Maly's Jahresber., Bd. 17, S. 115.

[4] *Ibid.*, S. 115.

[5] See foot-note 4, p. 139.

Absorption experiments with blood-serum have shown us further that the carbon dioxide which can be pumped out is in great part loosely chemically combined, and from this loose combination of the carbon dioxide it necessarily follows that the serum must also contain simply absorbed carbon dioxide. For the form of binding of the carbon dioxide contained in the serum or the plasma we find the three following possibilities: 1. A part of the carbon dioxide is simply absorbed; 2. Another part is loosely chemically combined; 3. A third part is in firm chemical combination.

The quantity of simply absorbed carbon dioxide has not been exactly determined. SETSCHENOW[1] considers the quantity in dog-serum to be about $\frac{1}{10}$ of the total quantity of carbon dioxide. According to the tension of the carbon dioxide in the blood and its absorption coefficient, the quantity seems to be still smaller.

The quantity of firmly chemically combined carbon dioxide in the blood-serum depends upon the quantity of simple alkali carbonate in the serum. This quantity is not known, and it cannot be determined either by the alkalinity found by titration, nor can it be calculated from the excess of alkali found in the ash, because the alkali is not only combined with carbon dioxide, but also with other bodies, especially with proteid. The quantity of firmly chemically combined carbon dioxide cannot be ascertained after pumping out *in vacuo* without the addition of acid, because to all appearances certain active constituents of the serum, acting like acids, expel carbon dioxide from the simple carbonate. The quantity of carbon dioxide not expelled from dog-serum by vacuum alone without the addition of acid amounts to 4.9 to 9.3 vols. per cent, according to the determinations of PFLÜGER.[2]

From the occurrence of simple alkali carbonates in the blood-serum it naturally follows that a part of the loosely combined carbon dioxide of the serum which can be pumped out must occur as bicarbonate. The occurrence of this combination in the blood-serum has also been directly shown. In experiments with the pump, as well as in absorption experiments, the serum behaves in other ways as a solution of bicarbonate, or carbonate of a corresponding concentration; and the behavior of the loosely combined carbon dioxide in the serum can be explained only by the occurrence of bicarbonate in the serum. By means of vacuum the serum always allows much more than one half of the carbon dioxide to be expelled, and it follows from this that in the pumping out not only may a dissociation of the bicarbonate take place, but also a conversion of the double sodium carbonate into a simple salt. As we know of no other carbon-dioxide combination besides the bicarbonate in the serum from which the carbon dioxide can be set free by simple dissociation *in vacuo*, we are obliged to assume that the serum must contain other faint acids, in addition to the carbon dioxide, which contend with it for the alkalies, and which expel the carbon dioxide from simple carbonates *in vacuo*. The carbon dioxide which is expelled by means of the pump and which, without regard to the simple absorbed quantity, is generally designated as "loosely chemically combined carbon dioxide," is thus only obtained in part in dissociable loose combination; in part it originates from the simple carbonates, from which it is expelled *in vacuo* by other faint acids.

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[1] Centralbl. f. d. med. Wissensch., 1877, No. 35.

[2] E. Pflüger, Ueber die Kohlensäure des Blutes. Bonn, 1864. S. 11. Cited from Zuntz in Hermann's Handbuch, S. 65.

These faint acids are thought to be in part phosphoric acid and in part globulins. The importance of the alkali phosphates for the carbon dioxide combination has been shown by the investigations of FERNET; but the quantity of these salts in the serum is, at least in certain kinds of blood, for example in ox-serum, so small that it can hardly be of importance. In regard to the globulins SETSCHENOW is of the opinion that they do not act as acids themselves, but form a combination with carbon dioxide, producing carboglobulinic acid, which unites with the alkali. According to SERTOLI,[1] whose views have lately found a supporter in TORUP, the globulins themselves are the acids which are combined with the alkali of the blood-serum. In both cases the globulins would form, directly or indirectly, that chief constituent of the plasma or of the blood-serum which, according to the law of the action of masses, contends with the carbon dioxide for the alkalies. By a greater partial pressure of the carbon dioxide the latter deprives the globulin alkali of a part of its alkali, and bicarbonate is formed; by low partial pressure the carbon dioxide escapes, and the bicarbonate is abstracted by the globulin alkali.

In the foregoing it has been assumed that the alkali is the most essential and important constituent of the blood-serum, as well as of the blood in general, in uniting with the carbon dioxide. The fact that the quantity of carbon dioxide in the blood greatly diminishes with a decrease in the quantity of alkali strengthens this assumption. Such a condition is found, for example, after poisoning with mineral acids. Thus WALTER[1] found only 2–3 vols. per cent carbon dioxide in the blood of rabbits into whose stomachs hydrochloric acid had been introduced. In the comatose state of diabetes mellitus the alkali of the blood seems to be in great part saturated with acid combinations, β-oxybutyric acid (STADELMANN,[2] MINKOWSKI), and MINKOWSKI[3] found only 3.3 vols. per cent carbon dioxide in the blood in diabetic coma.

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[1] Hoppe-Seyler, Med. chem. Untersuch.

In the above we have emphasized the fact that the oxygen in the blood occurs in a dissociable combination with the hæmoglobin, and that for the formation of this combination, oxyhæmoglobin, a distinct partial pressure of the oxygen is necessary for every variation in temperature. Also that the carbon dioxide of the blood, that which is contained in the blood-corpuscles as well as that in the plasma, occurs mostly in combinations which are dependent to a great extent upon the partial pressure of the carbon dioxide. Hence for the study of the exchange of gases between the blood and the alveolar air on one side, and the blood and the tissues on the other, special regard must be paid to the question as to how far this exchange of gases is the result of the law of diffusion and how far other forces take part in it; also the tension of the oxygen and the carbon dioxide is of the greatest importance. For these reasons it is best to treat of these questions in that section of this chapter dealing with the exchange of gas in the lungs and tissues.

Gases of the Lymph and Secretions.

The gases of the lymph are the same as in the blood-serum, and the lymph stands close to the blood-serum in regard to the quantity of the various gases, as well as to the kind of carbon dioxide combination. The investigations of DAENHARDT and HENSEN[4] on the gases of human lymph are at hand, but it still remains a question whether the lymph investigated was quite normal. The gases of normal dog-lymph were first investigated by the AUTHOR.[1] These gases contained traces of oxygen and consisted of 37.4–53.1% CO_2 and 1.6% N at 0° C. and 760 mm. Hg pressure. About one half of the carbon dioxide was firmly chemically combined. The quantity was greater than in the serum from arterial blood, but smaller than from venous blood.

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[1] Arch. f. exp. Path. u. Pharm., Bd. 7.

[2] *Ibid.*, Bd 17.

[3] Mittheil. a. d. med. Klink in Königsberg, 1888, and Arch. f. exp. Path. u. Pharm , Bd. 18.

[4] Virchow's Arch., Bd. 37.

The remarkable observation of BUCHNER[2] that the lymph collected after asphyxiation is poorer in carbon dioxide than that of the breathing animal is explained by ZUNTZ[3] by the formation of acid immediately after death in the tissues, and especially in the lymphatic glands, and this acid decomposes the alkali carbonates of the lymph in part.

The secretions with the exception of the saliva, in which PFLÜGER[4] and KÜLZ[5] found respectively 0.6 and 1% oxygen, are free from oxygen. The quantity of nitrogen is the same as in blood, and the chief mass of the gases consists of carbon dioxide. The quantity of this gas is chiefly dependent upon the reaction, i.e., upon the quantity of alkali. This follows from the analyses of PFLÜGER.[6] He found 19% carbon dioxide removable by the air-pump and 54% firmly combined carbon dioxide in a strongly alkaline bile, but on the contrary 6.6% carbon dioxide removable by the air-pump and 0.8% firmly combined carbon dioxide in a neutral bile. Alkaline saliva is also very rich in carbon dioxide. As average for two analyses made by PFLÜGER[7] of submaxillary saliva of a dog we have 27.5% carbon dioxide removable by the air-pump and 47.4% chemically combined carbon dioxide, making a total of 74.9%. KÜLZ[8] found a maximum of 65.78% carbon dioxide for the parotid saliva, of which 3.31% was removable by the air-pump and 62.47% was firmly chemically combined. From these and other statements on the quantity of carbon dioxide removable by the air-pump and chemically combined in the alkaline secretions it follows that bodies occur in them, although not in appreciable quantities, which are analogous to the albuminous bodies of the blood-serum and which act like faint acids.

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[1] Ber. d. k. sächs. Gesellsch. d. Wissensch., Math.-phys. Klasse, Bd. 23, 1871.

[2] Arbeiten a. d. physiol. Anstalt zu Leipzig, 1876.

[3] Hermann's Handbuch, Bd. 4, Thl. 2, S. 85.

[4] Pflüger's Arch., Bd. 1.

[5] Zeitschr. f. Biologie, Bd. 23.

[6] Pflüger's Arch., Bdd. 1 u. 2.

[7] L. c.

[8] L. c. It seems as if Külz's results were not calculated at 760 mm. Hg, but rather at 1 mm.

The acid or at any rate non-alkaline secretions, urine and milk, contain, on the contrary, considerably less carbon dioxide, which is nearly all removable by the air-pump, and a part seems to be loosely combined with the sodium phosphate. The figures found by PFLÜGER for the total quantity of carbon dioxide in milk and urine are 10 and 18.1–19.7% respectively.

EWALD[1] has made investigations on the quantity of gas in pathological transudations. He found only traces, or at least only very insignificant quantities, of oxygen in these fluids. The quantity of nitrogen was about the same as in blood. The quantity of carbon dioxide was greater than in the lymph (of dogs), and in certain cases even greater than the blood after asphyxiation (dog's blood). The tension of the carbon dioxide was greater than in venous blood. In exudations the quantity of carbon dioxide, especially that firmly combined, increases with the age of the fluid, while, on the contrary, the total quantity of carbon dioxide, and especially the quantity firmly combined, decreases with the quantity of pus-corpuscles.

II. The Exchange of Gas between the Blood on the one hand and Pulmonary Air and the Tissues on the Other.

In the introduction (Chapter I, p. 3) it was stated that we are to-day of the opinion, derived especially from the researches of PFLÜGER and his pupils, that the oxidations of the animal body do not take place in the fluids and juices, but are connected with the form-elements and tissues. It has, it is true, been shown by ALEX. SCHMIDT[2] and PFLÜGER[3] that oxidations take place in the blood, although only to a slight extent; but these oxidations depend, it seems, upon the form-elements of the blood, hence it does not contradict the above statement that the oxidations occur exclusively in the cells and chiefly in the tissues.

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[1] C. A. Ewald, Arch. f. Anat. u. Physiol., 1873 and 1876.

[2] Ber. d. k. sächs. Gesellsch. d. Wissensch., Math.-phys. Klasse, Bd. 19, 1867, and Centralbl. f. d. med. Wissensch., 1867, S. 356.

[3] Centralbl. f. d. med. Wissensch., 1867, S. 722.

The gaseous exchange in the tissues, which has been designated internal respiration, consists chiefly in that the oxygen passes from the blood in the capillaries to the tissues, while the chief bulk of the carbon dioxide of the tissues originates therein and passes into the blood of the capillaries. The exchange of gas in the lungs, which is called external respiration, consists, as we learn by a comparison of the inspired and expired air, in the blood taking oxygen from the air in the lungs and giving off carbon dioxide.

What kind of processes take part in this double exchange of gas? Is the gaseous exchange simply the result of an unequal tension of the blood on one side and the air in the lungs or tissues on the other? Do the gases pass from a place of higher pressure to one of a lower, according to the laws of diffusion, or are other forces and processes active?

These questions are closely related to another question as to the tension of the oxygen and carbon dioxide in the blood and in the air of the lungs and tissues.

Oxygen occurs in the blood in a disproportionately large part as oxyhæmoglobin, and the law of the dissociation of oxyhæmoglobin is of fundamental importance in the study of the tension of the oxygen in the blood.

If we recall that, according to **BOHR**, what we generally call oxyhæmoglobin is a mixture of hæmoglobins, which for one and the same oxygen pressure can unite with different quantities of oxygen, and also, as shown by **SIEGFRIED**, that there exists, besides the oxyhæmoglobin, another dissociable oxygen combination of hæmoglobin, namely, pseudohæmoglobin, it seems that we have several important preliminary questions to solve before we come to a discussion of the dissociation conditions of oxyhæmoglobin. As the above statements are in part contradicted and in part not sufficiently proven, and as also, according to **HÜFNER**, no difference exists between a oxyhæmoglobin solution and a solution of blood-corpuscles in regard to its delivery of oxygen, we are justified in setting the above statements aside for the present and only taking up the generally accepted and authoritative assertions.

For the understanding of the laws by which the oxygen is taken up by the blood in the alveoli of the lungs the investigations on the dissociation of oxyhæmoglobin are important, and especially those which relate to the dissociation at the temperature of the body are of great physiological importance. Several investigators have experimented on this subject, especially G. **HÜFNER**.[1] He has proven an important fact, namely, that a freshly prepared solution of pure oxyhæmoglobin crystals does not act unlike freshly defibrinated blood as regards the dissociation of oxyhæmoglobin. He also showed that the dissociation is dependent upon the concentration, namely, that at a given pressure a dilute solution is more strongly dissociated than a more concentrated solution. He found for solutions containing 14% oxyhæmoglobin that the dissociation at + 35° C. and an oxygen partial pressure of 75 mm. Hg was only very insignificant and only little stronger than with a partial pressure of 152 mm. In the first instance 96.89% of the total pigment was present as oxyhæmoglobin and 3.11% as hæmoglobin, while in the other case, at 152 mm. pressure, the respective figures were 98.42 and 1.58%. The dissociation becomes stronger first with an oxygen partial pressure of about 75 mm. Hg and downwards, and a corresponding increase in the quantity of reduced hæmoglobin; but even with an oxygen partial pressure of 50 mm. Hg the quantity of hæmoglobin was only 4.6% of the total pigment.

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[1] Du Bois-Reymond's Arch., 1890. Hüfner here gives also his older researches on this subject.

From these and older researches by HÜFNER[1] which were made at 35 or 39° C. it follows that the partial pressure of the oxygen may be reduced to one half of the atmospheric air without influencing essentially the quantity of oxygen in the blood or a corresponding solution of oxyhæmoglobin. We can also conclude from the quantity of oxygen or oxyhæmoglobin in the arterial blood that the tension of the oxygen in the arterial blood must be relatively higher. Based on the investigations of several experimenters, such as P. BERT,[2] HERTER,[3] and HÜFNER, who experimented partly on living animals and partly with hæmoglobin solutions, we generally consider the tension of the oxygen in arterial blood at the temperature of the body equal to an oxygen partial pressure of 75–80 mm. Hg.

We must now compare these figures with the tension of the oxygen in the air of the lungs.

Numerous investigations as to the composition of the inspired atmospheric air as well as the expired air are at hand, and we can say that these two kinds of air at 0° C. and a pressure of 760 mm. Hg have the following average composition in volume per cent:

	Oxygen.	Nitrogen.	Carbon Dioxide.
Atmospheric air........................	20.96	79.02	0.03
Expired air............................	16.03	79.59	4.38

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[1] L. c.

[2] Paul Bert, La pression barométrique. Paris, 1878.

[3] Zeitschr. f. physiol. Chem., Bd. 3.

The partial pressure of the oxygen of the atmospheric air corresponds at a normal barometric pressure of 760 mm. to a pressure of 159 mm. Hg. The loss of oxygen which the inspired air suffers in respiration amounts to about 4.93%, while the expired air contains about one hundred times as much carbon dioxide as the inspired air.

The expired air is therefore a mixture of alveolar air with the residue of inspired air remaining in the air-passages; hence in the study of the gaseous exchange in the lungs we must first consider the alveolar air. We have no direct determination of the composition of the alveolar air, but only approximate calculations. From the average results found by VIERORDT[1] in normal respiration for the carbon dioxide in the expired air, 4.63%, ZUNTZ[2] has calculated the probable quantity of carbon dioxide in the alveolar air as equal to 5.44%. If we start from this value, with the assumption that the quantity of nitrogen in the alveolar air does not essentially differ from the expired air, and admit that the quantity of oxygen in the alveolar air is 6% less than the inspired air, we find that the alveolar air contains 14.96% oxygen, corresponding to a partial pressure of 114 mm. Hg.

We have several direct determinations as to the composition of the alveolar air of dogs which show that the alveolar air is not much richer in carbon dioxide than the expired air.

By means of the lung catheter, an apparatus constructed by PFLÜGER, his pupils WOLFFBERG[3] and NUSSBAUM[4] have investigated the composition of the alveolar air of dogs. By the introduction of a catheter of a special construction into a branch of a bronchia the corresponding lobe of the lung may be hermetically sealed, while in the other lobes of the same lung and in the other lung the ventilation remains unhindered, so that no stowing of carbon dioxide takes place in the blood. When the cutting off lasts so long that a complete equalization between the gases of the blood and the cut-off air of the lungs is assumed, a sample of this air of the lungs is removed by means of the catheter and analyzed. In the air thus obtained from the lungs WOLFFBERG and NUSSBAUM found an average of 3.6% CO_2. NUSSBAUM has also determined the

[1] See ZUNTZ in Hermann's Handbuch, Bd. 4, Thl. 2, S. 105.

[2] Hermann's Handbuch, Bd. 4, Thl. 2, S. 106.

[3] Pflüger's Arch., Bdd. 5 u. 6.

[4] *Ibid.*, Bd. 7.

carbon-dioxide tension in the blood from the right heart in a case simultaneous with the catheterization of the lungs. He found nearly identical results, namely, a carbon-dioxide tension of 3.84% and of 3.81% of an atmosphere, which also shows that complete equalization between the gases of the blood and lungs in the enclosed parts of the lungs had taken place. According to these investigations a considerably higher oxygen partial pressure exists in the alveoli of the lungs than in the blood, and the taking up of oxygen from the air of the lungs is probably according to the laws of diffusion.

According to BOHR[1] the facts are otherwise, and the lungs, according to him, are active in the taking up of oxygen.

He experimented on dogs, allowing the blood, whose coagulation had been prevented by the injection of peptone solution or infusion of the leech, to flow from one bisected carotid to the other, or from the femoral artery to the femoral vein, through an apparatus called by him an hæmataërometer. The apparatus, which is a modification of LUDWIG's rheometer (stromuhr), allowed, according to BOHR, of a complete interchange between the gases of the blood circulating through the apparatus and a quantity of gas whose composition was known at the beginning of the experiment and enclosed in the apparatus. The mixture of gases was analyzed after an equalization of the gases by diffusion. In this way the tension of the oxygen and carbon dioxide in the circulating arterial blood was determined. During the experiment the composition of the inspired and expired air was also determined, the number of inspirations noted, and the extent of respiratory exchange of gas measured. To be able to compare between the gas tension in the blood and in an expiration air, whose composition was closer to the unknown composition of the alveolar air than the ordinary expired air, the composition of the expired air at the moment it passed the bifurcation of the trachea was ascertained by special calculation. The tension of the gases in this "bifurcated air" could be compared with the tension of the gases of the blood, and in such a way that the comparison in both cases took the same time.

BOHR found remarkably high results for the oxygen tension in arterial blood in this series of experiments. They varied between 101 and 144 mm. Hg pressure. In eight out of nine experiments on the breathing of atmospheric air, and in four out of five experiments on breathing air containing carbon dioxide, the oxygen tension in the arterial blood was higher than the "bifurcated air." The greatest difference, where the oxygen tension was higher in the blood than in the air of the lungs, was 38 mm. Hg.

According to BOHR we cannot simply explain the taking up of oxygen by the blood from the air of the lungs by a higher partial pressure of the oxygen. The difference in tension between the two sides of the walls of the alveoli may therefore, according to him, not be the only force which serves in the migration of the oxygen through the lung tissue, and the lungs themselves must, according to Bohr, exercise an unknown specific action in the taking up of oxygen.

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[1] Skand. Arch. f. Physiol., Bd. 2.

Hüfner[1] has made the objection to Bohr's views that in the experimental conditions established by Bohr the equilibrium between the air in the apparatus and the gases of the blood had not probably set in. Frédericq[2] has also come to the same conclusion by experiments with uncoagulable, living arterial dog's blood, allowing it to flow through an aërotonometer-tube (see Tension of Carbon Dioxide), whereby a very slow diffusion equalization took place between the gases of the circulating blood and the air enclosed in the tube. When the original partial pressure of the oxygen in the aërotonometer atmosphere was very low or very high the diffusion equilibrium was not reached inside of an hour. Frédericq also found that the oxygen tension in arterial peptone blood of the dog remained always several per cent of an atmosphere below the partial pressure of the oxygen in the air of the lung alveoli. We have therefore no sufficient ground to abandon the present generally accepted view, that the oxygen is taken up in the lungs simply by diffusion.

As the hæmoglobin obtained from different blood portions does not, according to Bohr, always take up the same quantity of oxygen for each gramme, so, according to him, the hæmoglobin within the blood-corpuscle may show a similar behavior. He calls the quantity of oxygen (measured at 0° C. and 760 mm. Hg) which is taken up by 1 grm. hæmoglobin of the blood at 15° C. and an oxygen pressure of 150 mm. the *specific oxygen capacity*.[3] This quantity, according to Bohr, may be different not only in different individuals, but also in the different vascular systems of the same animal, and it may also be changed experimentally by bleeding, breathing air deficient in oxygen, or poisoning. It is now evident that one and the same quantity of oxygen in the blood, other things being equal, must have a different tension according to the specific oxygen capacity is greater or smaller. The tension of the oxygen may, according to Bohr, be changed without changing the quantity of oxygen, and the animal body must, according to him, have means of varying the tension of the oxygen in the tissues in a short time without changing the quantity of oxygen contained in the blood. The great importance of such a property of the tissues for respiration is evident; but it is perhaps too early to give a positive opinion on Bohr's statements and experiments.

It is to be inferred, from the above statements in regard to the tension and dissociation of the oxygen of the blood, that the quantity of oxygen in the blood is not essentially dependent upon the quantity of oxygen in the air, at least within certain limits. This in fact is the case.

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[1] Du Bois-Reymond's Arch., 1890, S. 10.

[2] Centralbl. f. Physiologie, Bd. 7, S. 33.

[3] Centralbl. f. Physiol., Bd. 4, S. 254.

That the raising of the oxygen pressure, even to a pressure of one atmosphere, has no essential influence on the quantity of oxygen taken up and on the carbon dioxide eliminated, has been known for a long time (Lavoisier, Regnault, and Reiset[1]). Further experiments in this direction have been made by Paul Bert.[2] He found that in pure oxygen at a pressure of 3 atmospheres, or in ordinary air at a pressure of 15 atmospheres, animals quickly died with convulsions. Before and during the spasms a lowering of temperature took place, and the consumption of oxygen, as well as the elimination of carbon dioxide and the combustion of the sugar of the blood, was lowered. By raising the oxygen pressure of the air to 3 atmospheres the quantity of oxygen contained in the blood is somewhat increased. It seems that the quantity of oxygen which is here taken up corresponds to that quantity which is simply absorbed by the blood at that pressure.

It is also of special interest to know to what extent the partial pressure of the oxygen of the air can be lowered without causing any injurious action or danger to life. A great many observations have been made on this subject, partly on man and partly on animals. It follows from these observations that this limit may undergo considerable variation. In human beings it seems to be somewhat higher than in certain animals, the rabbit for example. P. Bert[3] found on experiments on himself in diluted air that a gas mixture with 11.3% oxygen caused serious disturbance. Leblanc[4] found no difficulty in breathing air containing 15.3%, but with 9.8% oxygen he felt dizzy, nauseated, and faint. The aeronauts Sivel and Crocé-Spinelli[5] died at an air-pressure of 260 mm. Hg, corresponding to 7.2% oxygen.

The statements of Loewy[6] are of special interest in regard to breathing under diminished oxygen-pressure. In the person experimented upon the minimum alveolar oxygen-tension sufficient for the normal processes of metabolism was equal to 40–45 mm. Hg, which according to Hüfner's table corresponds to a mixture of about 94% oxyhæmoglobin and 6% hæmoglobin. This minimum alveolar oxygen-tension may be reached, according to LOEWY, with different quantities of oxygen in the inspired air by changing the breathing mechanisms. By greatly increasing the quantity of inspired air in a unit of time, as by a deeper inspiration, the alveolar oxygen-tension may remain constant or even be increased, namely, on lowering the oxygen-tension. Thus LOEWY observed in an experiment an alveolar oxygen-tension of 41.2 mm. Hg with 12.2% oxygen in the inspired air, 6.14 litres respired air per minute, and 292.6 c. c. the volume of each inspiration. In two other experiments, where the volume of the respired air was 31.4 and 35.9 litres per minute with 785 and 972 c. c. for every inspiration, the alveolar oxygen-tension was 43.9 and 43.4 mm., with 7.522 and 7.32% oxygen in the inspired air. Lowering the alveolar oxygen-tension to the limit 40–45 mm. caused no change on the breathing mechanisms and did not correspondingly change the respiratory quotient. Below this limit the gaseous exchange is so changed that the elimination of carbon dioxide as compared with the taking up of oxygen increases and the respiratory quotient is correspondingly raised.

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[1] See Hoppe-Seyler, Physiol. Chem., S. 545.

[2] La pression barométrique. Paris, 1878.

[3] L. c.

[4] Cited from P. Bert, La pression barométrique.

[5] Cited from Hoppe-Seyler, Physiol. Chem., S. 9 u. 549.

[6] Pflüger's Arch., Bd. 58.

In certain animals the limit seems to be lower than with human beings. W. MÜLLER,[1] FRIEDLÄNDER and HERTER[2] noted this in rabbits. With 7–5% oxygen in the inspired air a strong dyspnœa occurred in the experiments of FRIEDLÄNDER and HERTER, but the animal, which breathed under a large receiver, did not die until 1½–2 hours after the quantity of oxygen had sunk to 2.1–3.8%. HOPPE-SEYLER and STROGANOW[3] found that in dogs the respiratory movement stopped when the quantity of oxygen in the respired air had sunk to 3.542%, and BERT found in experiments on different animals, including frogs and birds, that death occurred when the quantity of oxygen was from 1.3 (in frogs) to 4.4%.

In regard to the quantity of oxygen in the blood at lower air-pressures, the observations of FRÄNKEL and GEPPERT[4] on dogs must be mentioned. At an air-pressure of 410 mm. mercury the quantity of oxygen in the arterial blood was normal; at an air-pressure of 378–365 mm. it was a little diminished, and only at a lowering of the pressure to 300 mm. was a considerable decrease of the oxygen observed.

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[1] Wien. Sitzungsber., Bd. 33, and Annal. d. Chem. u. Pharm., Bd. 108.

[2] Zeitschr. f. physiol. Chem., Bd. 3.

[3] Pflüger's Arch., Bd. 12.

[4] Ueber die Wirkungen der verdünnten Luft auf den Organismus. Berlin, 1883.

The question how it is possible for man and animals to live for any length of time in high altitudes with a diminished oxygen pressure is important in this connection. In this regard Viault[1] has called attention to the fact that the number of blood-corpuscles is greater in such individuals. Thus the llama, according to him, has about 16 million blood-corpuscles per cubic millimetre. By observations on himself and other persons, as well as on animals, Viault found the first effect of living in high altitudes is a considerable increase in the number of red corpuscles, which in his own case amounted to 5–8 million. The quantity of hæmoglobin is on the contrary, according to Viault, increased only in narrow limits on living for some time in the mountains, but the hæmoglobin is divided among so many more blood-corpuscles, and therefore a much greater surface comes in contact with oxygen. Contrary to the statement of Viault, Müntz[2] has found that among the above-mentioned conditions a considerable increase in the quantity of iron and hæmoglobin in the blood takes place. Egger[3] found among the influences of high climates a considerable increase in the number of blood-corpuscles as well as in the quantity of hæmoglobin, while Koeppe,[4] on the contrary, observed a diminution of the latter besides a great increase in the number of blood-corpuscles. Regnard[5] has observed a considerable increase in the quantity of hæmoglobin in a guinea-pig which was enclosed in a receiver for a whole month with a diminution of pressure corresponding to a height of 3000 metres.

The tension of the carbon dioxide in the blood has been determined in different ways by Pflüger and his pupils, Wolffberg,[6] Strassburg,[7] and Nussbaum.[8] According to the aërotonometric method the blood is allowed to flow directly from the artery or vein through a glass tube which contains a gas mixture of a known composition If the tension of the carbon dioxide in the blood is greater than the gas mixture, then the blood gives up carbon dioxide, while in the reverse case it takes up carbon dioxide from the gas mixture. The analysis of the gas mixture after passing the blood through it will also decide if the tension of the carbon dioxide in the blood is greater or less than in the gas mixture; and by a sufficiently great number of determinations, especially when the quantity of carbon dioxide of the gas mixture corresponds as nearly as possible in the beginning to the probable tension of this gas in the blood, we may learn the tension of the carbon dioxide in the blood.

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[1] Compt. rend., Tomes 111, 112 et 114.

[2] Compt. rend., Tome 112.

[3] Cited from Maly's Jahresber., Bd. 23.

[4] *Ibid.*, Bd. 23.

[5] Compt. rend. Soc. de Biol., 1892. Cited from Centralbl. f. Physiologie, Bd. 7, 1893.

[6] Pflüger's Arch., Bd. 6.

[7] *Ibid.*, Bd. 6.

[8] *Ibid.*, Bd. 7.

According to this method the carbon-dioxide tension of the arterial blood is on an average 2.8% of an atmosphere, corresponding to a pressure of 21 mm. mercury (STRASSBURG[1]). In the blood from the pulmonary alveoli NUSSBAUM[2] found a carbon-dioxide tension of 3.81% of an atmosphere, corresponding to a pressure of 28.95 mm. mercury. STRASSBURG, who experimented on tracheotomized dogs in which the ventilation of the lungs was less active and therefore the carbon dioxide was removed from the blood with less readiness, found in the venous blood of the heart a carbon-dioxide tension of 5.4% of an atmosphere, corresponding to a partial pressure of 41.01 mm. mercury.

Another method is the catheterization of a lobe of the lungs (see page 595). In the air thus obtained from the lungs NUSSBAUM and WOLFFBERG found an average of 3.6% CO_2. NUSSBAUM, as previously mentioned, has also determined the carbon-dioxide tension in the blood of the pulmonary alveoli in a case simultaneous with the catheterization of the lungs. He found nearly identical results, namely, a carbon-dioxide tension of 3.84% and 3.81%.

BOHR in his experiments above mentioned (page 596) has arrived at other results in regard to the carbon-dioxide tension. In eleven experiments with inhalation of atmospheric air the carbon-dioxide tension in the arterial blood varied from 0 to 38 mm. Hg, and in five experiments with inhalation of air containing carbon dioxide from 0.9 to 57.8 mm. Hg. A comparison of the carbon-dioxide tension in the blood with the bifurcated air gave in several cases a greater carbon-dioxide pressure in the air of the lungs than in the blood, and as maximum this difference amounted to 17.2 mm. in favor of the air of the lungs in the experiments with inhalation of atmospheric air. As the alveolar air is richer in carbon dioxide than the bifurcated air, this experiment unquestionably proves, according to BOHR, that the carbon dioxide has migrated against the high pressure.

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[1] L. c. [2] L. c.

In opposition to these investigations, FRÉDERICQ[1] in his above-mentioned experiments, obtained the same figures for the carbon-dioxide tension in arterial peptone blood as PFLÜGER and his pupils found for normal blood. The low figures obtained by BOHR for the carbon-dioxide tension appear remarkable when we recall that GRANDIS[2] found in peptone blood which LAHOUSSE[3] and BLACHSTEIN[4] had shown was poor in carbon dioxide a high carbon-dioxide tension.

A certain importance has been ascribed to oxygen in regard to the elimination of carbon dioxide in the lungs, in that it has an expelling action on the carbon dioxide from its combinations in the blood. This statement, first made by HOLMGREN,[5] has recently found an advocate in WERIGO.[6] This investigator has made ingenious experiments on living animals in which he allows both lungs of the animal to breathe separately, the one with hydrogen and the other with pure oxygen or a gas mixture rich in oxygen. He invariably found a greater carbon-dioxide tension in the air sucked from the lungs in the presence of oxygen, and he draws the conclusion from his experiments that the oxygen passing from the lung alveoli into the blood raises the carbon-dioxide tension. According to WERIGO, by this action the oxygen is a powerful factor in the elimination of carbon dioxide, and therefore it is not necessary to assume a specific action of the lung itself in these processes.

ZUNTZ[7] has suggested important objections to the investigations of WERIGO, but they have not been substantiated by experiment; hence the question is still open.

Also in regard to the elimination of carbon dioxide in the lungs we have no striking reason for abandoning the common view that the carbon dioxide simply passes from the blood into the air of the lungs according to the laws of diffusion.

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[1] Centralbl. f. Physiol., Bd. 7.

[2] Du Bois-Reymond's Arch., 1891, S. 499.

[3] *Ibid.*, 1889, S. 77.

[4] *Ibid.*, 1891, S. 394.

[5] Wien. Sitzungsber., Math.-nat. Klasse, Bd. 48.

[6] Pflüger's Arch., Bdd. 51 u. 52.

[7] *Ibid.*, Bd. 52.

From what has been said above (page 593) in regard to the internal respiration we derive that it consists chiefly in that in the capillaries the oxygen passes from the blood into the tissues, while the carbon dioxide passes from the tissues into the blood.

The assertion of ESTOR and SAINT PIERRE[1] that the quantity of oxygen in the blood of the arteries decreases with the remoteness from the heart has been shown as incorrect by PFLÜGER,[2] and the oxygen tension in the blood on entering the capillaries must be higher. As compared with the capillaries the tissues are to be considered as nearly or entirely free from oxygen, and in regard to the oxygen a considerable difference in pressure must exist between the blood and tissues. The possibility that this difference in pressure is sufficient to supply the tissues with the necessary quantity of oxygen is hardly to be doubted.

In regard to the carbon-dioxide tension in the tissue we must assume *a priori* that it is higher than in the blood. This is found to be true. STRASSBURG[3] found in the urine of dogs and in the bile a carbon-dioxide tension of 9% and 7% of an atmosphere, respectively. The same experimenter has, further, injected atmospheric air into a ligatured portion of the intestine of a living dog and analyzed the air taken out after some time. He found a carbon-dioxide tension of 7.7% of an atmosphere. The carbon-dioxide tension in the tissues is considerably greater than in the venous blood, and there is no objection to the view that the carbon dioxide simply diffuses from the tissues to the blood according to the laws of diffusion.

That a true secretion of gases occurs in animals follows from the composition and behavior of the gases in the swimming-bladder of fishes. These gases consist of oxygen and nitrogen with only small quantities of carbon dioxide. In fishes which do not live at any great depth the quantity of oxygen is ordinarily as high as in the atmosphere, while fishes which live at great depths may, according to BIOT and others,[4] contain considerably more oxygen and even above 80%. MOREAU[5] has also found that after emptying the swimming-bladder by means of a trocar new air collected after a time, and this air was richer in oxygen than the atmospheric air and contained even 85% oxygen. BOHR,[6] who has proved and confirmed these statements, also found that this collection is under the influence of the nervous system, because on

[1] Journ. de l'anat. de la physiol., Tome 2, 1865.

[2] Pflüger's Arch., Bd. 1.

[3] Pflüger's Arch., Bd. 6.

[4] See Hermann's Handb., Bd. 4, Thl. 2, S. 151.

[5] Compt. rend., Tome 57, S. 37 u. 816.

[6] Journ. of Physiol., Vol. 15. See also Hüfner, Du Bois-Reymond's Arch., 1892.

the section of certain branches of pneumogastric nerve it is discontinued. It is beyond dispute that we have here a secretion and not a diffusion of oxygen.

Several methods have been suggested for the study of the quantitative relationship of the respiratory exchange of gas. We must refer the reader to other text-books for more details of these methods, and we will only here mention the chief traits of the most important methods.

Regnault and Reiset's *Method.* According to this method the animal or person experimented upon is allowed to breathe in an enclosed space. The carbon dioxide is removed from the air, as it forms, by strong caustic alkali, from which the quantity may be determined, while the oxygen is replaced continually by exactly measured quantities. This method, which also makes possible a direct determination of the oxygen used as well as the carbon dioxide produced, has since been modified by other investigators, such as Pflüger and his pupils, Seegen and Nowak, and Hoppe-Seyler.[1]

Pettenkofer's *Method.*[2] According to this method the individual to be experimented upon breathes in a room through which a current of atmospheric air is passed. The quantity of air passed through is carefully measured. As it is impossible to analyze all the air made to pass through the chamber, a small fraction of this air is diverted into a branch line during the entire experiment, carefully measured, and the quantity of carbon dioxide and water determined. From the composition of this air the quantity of water and carbon dioxide contained in the large quantity of air made to pass through the chamber can be calculated. The consumption of oxygen cannot be directly determined in this method, but may be indirectly by difference, which is a defect in this method.

Speck's *Method.*[3] For briefer experiments on man Speck has used the following: He breathes into two spirometer receivers on which the gas volume can be read off very accurately, through a mouthpiece with two valves, closing the nose with a clamp. The air from one of the spirometers is inhaled through one valve, and the expired air passes through the other into the other spirometer. By means of a rubber tube connected with the expiration tube an accurately measured part of the expired air may be passed into an absorption tube and analyzed.

Zuntz and Geppert's *Method.*[4] This method, which has been improved by Zuntz and his pupils from time to time, consists in the following: The individual being experimented upon inspires pure atmospheric air through a very wide feed-pipe leading from the open air, the inspired and the expired air being separated by two valves (human subjects breathe with closed nose by means of a soft rubber mouthpiece, animals through an air-tight tracheal canula). The volume of the expired air is measured by a gas-meter, and an aliquot part of this air collected and the quantity of carbon dioxide and oxygen determined. As the composition of the atmospheric air can be considered as constant within a certain limit, the production of carbon dioxide as well as the consumption of oxygen may be readily calculated (see the works of Zuntz and his pupils).

Hanriot and Richet's *method*[5] is characterized by its simplicity. These investigators allow the total air to pass through three gas-meters, one after the other. The first measures the inspired air, whose composition is known. The second gas-meter measures the expired air, and the third the quantity of the expired air after the carbon dioxide has been removed by a suitable apparatus. The quantity of carbon dioxide produced and the oxygen consumed can be readily calculated from these data.

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[1] In regard to this method see Zuntz in Hermann's Handb., Bd. 4, Thl. 2, and Hoppe-Seyler in Zeitschr. f. physiol. Chem., Bd. 19.

[2] See Zuntz, l. c.

[3] Speck, Physiologie des menschlichen Athmens. Leipzig, 1892.

[4] Pflüger's Arch., Bd. 42. See also Magnus-Levy in Pflüger's Arch., Bd. 55, S. 10, in which the work of Zuntz and his pupils is cited.

[5] Compt. rend., Tome 104.

Appendix.

The Lungs and their Expectorations.

Besides *proteid bodies* and the *albuminoids* of the connective substance group, *lecithin*, *taurin* (especially in ox-lungs), *uric acid*, and *inosit* have been found in the lungs. POULET[1] claims to have found a special acid, which he has called pulmotartaric acid, in the lung tissue. Glycogen occurs abundantly in the embryonic lung, but is absent in the adult lung.

The black or dark brown pigment in the lungs of human beings and domestic animals consists chiefly of carbon, which originates from the soot in the air. The pigment may in part also consist of melanin. Besides carbon, other bodies, such as iron oxide, silicic acid, and clay, may be deposited in the lungs, being inhaled as dust.

Among the bodies found in the lungs under pathological conditions we must specially mention albumoses and peptones (in pneumonia and suppuration), glycogen, a faintly dextrorotatory carbohydrate differing from glycogen found by POUCHET[2] in consumptives, and finally also cellulose, which, according to FREUND,[3] occurs in the lungs, blood, and pus of persons with tuberculosis.

C. W. SCHMIDT[4] found in 1000 grms. mineral bodies from the normal human lung the following: NaCl 130, K_2O 13, Na_2O 195, CaO 19, MgO 19, Fe_2O_3 32, P_2O_5 485, SO_3 8, and sand 134 grms. According to OIDTMANN[5] the lungs of a 14-day-old child contained 796.05 p. m. water, 198.19 p. m. organic bodies, and 5.76 p. m. inorganic bodies.

The expectoration is a mixture of the mucous secretion of the respiratory passages, of saliva and buccal mucus. Because of this its composition is very variable, especially under pathological conditions when various products mix with it. The chemical constituents are, besides the mineral substances, chiefly mucin with a little proteid and nuclein substance. Under pathological conditions albumoses and peptones, volatile fatty acids, glycogen, CHARCOT'S crystals, and also crystals of cholesterin, hæmatoidin, tyrosin, fat, and fatty acids, triple phosphates, etc., have been found.

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[1] Cited from Maly's Jahresber., Bd. 18, S. 248.

[2] Compt. rend., Bd. 96.

[3] Wien. med. Jahrb., 1886. Cited from Maly's Jahresber., Bd. 16, S. 471.

[4] Cited from v. Gorup-Besanez, Lehrbuch, 4. Aufl., S. 727.

[5] *Ibid.*, S. 732.

The form constituents are, under physiological circumstances, epithelium-cells of various kinds, leucocytes, sometimes also red blood-corpuscles and various kinds of fungi. In pathological conditions elastic fibres, spiral formations consisting of a mucin-like substance, fibrin coagulum, pus, pathogenic microbes of various kinds, and the above-mentioned crystals occur.

CHAPTER XVIII.

METABOLISM[1] WITH VARIOUS FOODS, AND THE DEMAND FOR FOOD IN MAN.

THE conversion of chemical tension into living energy, which characterizes animal life, leads, as previously stated in Chapter I, to the formation of relatively simple compounds—carbon dioxide, urea, etc.—which leave the organism, and which, moreover, being very poor in potential energy, are for this reason of no or very little value for the body. It is therefore absolutely necessary for the continuance of life and the normal course of the functions of the body that the organism and its different tissues should be supplied with new material to replace that which has been exhausted. This is accomplished by means of food. Those bodies are designated as *food* which have no injurious action upon the organism and which replace those constituents of the body that have been consumed in metabolism or that can prevent or diminish the consumption of such constituents.

Among the numerous dissimilar substances which man and animals take with the food all cannot be equally necessary or have the same value. Some perhaps are unnecessary, while others may be indispensable. We have learned by direct observation and a wide experience that besides the oxygen, which is necessary for oxidation, the essential foods for animals in general, and for man especially, are *water, mineral bodies, proteids, carbohydrates,* and *fats.*

It is also apparent that the various groups of food-stuffs necessary for the tissues and organs must be of different importance; thus, for instance, water and the mineral bodies have another value than the organic foods, and these again must vary in importance among themselves. The knowledge of the action of various nutritive bodies on the exchange of material from a qualitative as well as a quantitative point of view must be of fundamental importance in determining the value of different nutritive substances relative to the demands of the body for food under various conditions and also in deciding many other questions—for instance, the proper nutrition for an individual in health and in disease.

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[1] The translator will use in the following pages for the German word "*Stoffwechsel*" Dr. Burdon-Sanderson's translation (Syllabus of Lectures, 1879). *exchange of material,* and at the same time the more general term "*metabolism.*"

Such knowledge can only be attained by a series of systematic and thorough observations, in which the quantity of nutritive material, relative to the weight of the body, taken and absorbed in a given time is compared with the quantity of final metabolic products which leave the organism at the same time. Researches of this kind have been made by several investigators, but above all should be mentioned those made by BISCHOFF and VOIT, by PETTENKOFER and VOIT, and by VOIT and his pupils.

It is absolutely necessary in researches on the exchange of material to be able to collect, analyze, and quantitatively estimate the excreta of the organism, so that they may be compared with the quantity and composition of the nutritive bodies taken up. In the first place, one must know what the habitual excreta of the body are and in what way these bodies leave the organism. One must also have trustworthy methods for the quantitative estimation of the same.

The organism may, under physiological conditions, be exposed to accidental or periodic losses of valuable material—such losses as only occur in certain individuals, or in the same individual only at a certain period ; for instance, the secretion of milk, the production of eggs, the ejection of semen or menstrual blood. It is therefore apparent that these losses can only be the subject of investigation and estimation in special cases.

The regular and constant excreta of the organism are of the very greatest importance in the study of metabolism. To these belong, in the first place, the true final metabolic products—CARBON DIOXIDE, UREA (uric acid, hippuric acid, creatinin, and other urinary constituents), and a part of the WATER. The remainder of the water, the mineral bodies, and those secretions or tissue-constituents —MUCUS, DIGESTIVE FLUIDS, SEBUM, SWEAT, and EPIDERMIS FORMATIONS—which are either poured into the intestinal tract, or secreted from the surface of the body, or broken off and thereby lost for the body, also belong to the constant excreta.

The remains of food, sometimes indigestible, sometimes digestible but not acted upon, contained in the fæces, which vary considerably in quantity and composition with the nature of the food, also belong to the excreta of the organism. Even though these remains, which are never absorbed and therefore are never constituents of the animal fluids or tissues, cannot be considered as excreta of the body in a strict sense, still their quantitative estimation is absolutely necessary in certain experiments on the exchange of material.

The determination of the constant loss is in some cases accompanied with the greatest difficulties. The loss from the detached epidermis, from the secretion of the sebaceous glands, etc., cannot be determined with exactness without difficulty, and therefore—as they do not occasion any mentionable loss because of their small quantity—they need not be considered in quantitative experiments on metabolism. This also applies to the constituents of the mucus, bile, pancreatic and intestinal juices, etc., occurring in the contents of the intestine, and which, leaving the body with the fæces, cannot be separated from the other contents of the intestine and therefore cannot be quantitatively determined separately. The uncertainty which, because of the intimated difficulties, attaches itself to the results of the experiments is very small as compared to the variation which is caused by different individualities, different modes of living, different foods, etc. No general but only approximate values can therefore be given for the constant excreta of the human body.

The following figures represent the quantity of excreta for 24 hours of a grown man weighing 60–70 kilos on a mixed diet. The numbers are compiled from the results of different investigators.

	Grammes.
Water	2500–3500
Salts (with the urine)	20–30
Carbon dioxide	750–900
Urea	20–40
Other nitrogenous urinary constituents	2–5
Solids in the excrements	30–50

These total excreta are approximately divided among the various excretions in the following way—but still it must not be forgotten that this division may vary to a great extent under various external circumstances: by RESPIRATION about 32%, by the EVAPORATION FROM THE SKIN 17%, with the URINE 46–47%, and with the EXCREMENTS 5–9%. The elimination by the skin and lungs, which is sometimes differentiated by the name "PERSPIRATIO INSENSIBILIS" from the visible elimination by the kidneys and intestine, is on an average about 50% of the total elimination. This proportion, only quoted relatively, is subject to considerable variation, because of the great difference in the loss of water through the skin and kidneys under different circumstances.

About 90% of the water in carnivora is excreted through the kidneys. In herbivora 60% of the water is eliminated by the excrements, which are 30–50% of the total excreta. In man only a smaller fraction of the water (about 5%) is eliminated with the fæces, and the great mass of the water is divided between the kidneys, lungs, and skin.

The nitrogenous constituents of the excretions consist chiefly of urea, or uric acid in certain animals, and the other nitrogenous urinary constituents. A disproportionally large part of the nitrogen leaves the body with the urine, and, as the nitrogenous constituents of this excretion are final products of the metabolism of proteids in the organism, the quantity of proteids transformed in the body may be easily calculated by multiplying the quantity of nitrogen in the urine by the coefficient 6.25 ($\frac{100}{16} = 6.25$), if we admit that the proteids contain in round numbers 16% nitrogen.

Still another question is whether the nitrogen leaves the body only with the urine or by other channels. This last is habitually the case. The discharges from the intestine always contain some nitrogen which has a twofold origin. A part of this nitrogen depends upon undigested or non-absorbed remnants of food, and another part on the non-absorbed remains of digestive secretions—bile, pancreatic juice, intestinal mucus—and of epithelium-cells of the mucous membrane. It follows that a part of the nitrogen of fæces has this last-mentioned origin from the fact that discharges from the intestine occur also in complete inanition.

If the question to be decided is, how much of the nitrogenous bodies is assimilated in certain modes of nutrition or after taking a certain quantity of food, then naturally the quantity of nitrogen originating from the food and leaving the body with the excrements must be subtracted from the total quantity of nitrogen taken up with the food. To obtain the quantity of nitrogen leaving the body with the excrements it is necessary to subtract from the total quantity of nitrogen of the excrements the quantity of nitrogen coming from the digestive tract itself and its secretions, and the amount of this last must be known.

It is obvious that exact results which answer for all times cannot be given for that part of the nitrogen which has its origin in the digestive canal and fluids. It may not only vary in different individuals, but also in the same individual after more or less active secretion and absorption. In the attempts made to determine this part of the nitrogen of the excrements it has been found that in man, on non-nitrogenous or nearly nitrogen-free food, it amounts in round numbers to somewhat less than 1 grm. per 24 hours (RIEDER,[1] RUBNER.[2]) During starvation, in which a smaller quantity of digestive secretions is eliminated, it is less. MÜLLER[3] found in his observations on the faster CETTI that only 0.2 grm. nitrogen was derived from the intestinal canal.

Nitrogen also leaves the body through the horn formation. The quantity which is lost in this manner is, though it cannot be exactly determined, insignificant. Man loses only about 0.03 grm. nitrogen daily by means of the hair and nails (MOLESCHOTT[4]) and about 0.3–0.5 grm. by the scaling off of the skin. The quantity of nitrogen which leaves the body under ordinary circumstances by the perspiration must be so small that, like the loss by the horny structure, it need not be considered in experiments on the exchange of material. The elimination of nitrogen by the perspiration need only be considered in cases of profuse sweating.

The view was formerly held that in man and carnivora an elimination of gaseous nitrogen took place through the skin and lungs, and because of this, on comparing the nitrogen of the food with that of the urine and fæces, a *nitrogen deficit* occurred in the visible elimination.

This question has been the subject of much discussion and of numerous investigations. The conclusion has been drawn from the respiration researches of REGNAULT and REISET[5] that also an exhalation of nitrogen takes place. SEEGEN and NOWAK[6] especially have recently endeavored to prove the correctness of this conclusion. Such an experiment is, however, accompanied with so many difficulties, and there are so many sources of error, that it can scarcely be considered as conclusive. In fact, PETTENKOFER and VOIT[1] have demonstrated the existence of errors in the experiments of SEEGEN and NOWAK. On the other hand, PFLÜGER and LEO[2] have found no appreciable exhalation of nitrogen in rabbits.[3] Also many investigators, especially PETTENKOFER and VOIT,[4] have shown by experiments on man and animals that with the proper quantity and quality of food we can bring the body into *nitrogenous equilibrium*, in which the quantity of nitrogen voided with the urine and fæces is equal or nearly equal to the quantity contained in the food.

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[1] Zeitschr. f. Biologie, Bd. 20.

[2] *Ibid.*, Bd. 15.

[3] Berl. klin. Wochenschr., 1887.

[4] Untersuch. zur Naturlehre, Bd. 12.

[5] Annal. d. Chim. et Phys. (3), Tome 27, and Annal. d. Chem. u. Pharm., Bd. 73.

[6] Wien. Sitzungsber., Bd. 71, and Pflüger's Arch., Bd. 25.

The experiments made by GRUBER in VOIT'S institute seem to be especially conclusive on this point. GRUBER[5] fed a dog seventeen days on meat which in all contained 368.53 grms. nitrogen, and he found in the same time 368.28 grms. nitrogen in the urine and fæces. In later experiments[6] he found a difference varying only between $-$ 0.21% and $+$ 1%. From this and other experiments we may conclude with VOIT that a deficit of nitrogen does not exist; or it is so insignificant that in experiments upon metabolism it need not be considered. In investigations on the metabolism of proteids in the body, ordinarily, it is only necessary to consider the nitrogen of the urine and fæces, but it must be remarked that the nitrogen of the urine is a measure of the extent of the metabolism of the proteids in the body, while the nitrogen of the fæces (after deducting somewhat less than 1 grm. on mixed diet) is a measure of the non-absorbed part of the nitrogen of the food.

In the oxidation of the proteids in the organism their sulphur is oxidized into sulphuric acid, and on this depends the fact that the elimination of sulphuric acid by the urine, which in man is only to a small extent derived from the sulphates of the food, makes nearly equal variations as the elimination of nitrogen by the urine. If we consider the amount of nitrogen and sulphur in the proteids as 16% and 1% respectively, then the proportion between the nitrogen of the proteids and the sulphuric acid, H_2SO_4, produced by their burning is in the ratio 5.2 : 1, or about the same as in the urine (see page 515). The determination of the quantity of sulphuric acid eliminated with the urine gives us an important means of controlling the extent of the transformation of proteids, and such a control is especially important in cases in which we wish to study the action of certain nitrogenous non-albuminous bodies on the metabolism of proteids. A determination of the nitrogen alone is not sufficient in such cases.

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[1] Zeitschr. f. Biologie, Bd. 16.

[2] Pflüger's Arch., Bd. 26.

[3] Zuntz und Tacke, Maly's Jahresber., Bd. 16, S. 361.

[4] See Voit in Hermann's Handbuch, Bd. 6, Th. 1.

[5] Zeitschr. f. Biologie, Bd. 16.

[6] *Ibid.*, Bd. 19.

If it is found, on comparing the nitrogen of the food with that of the urine and fæces, that there is an excess of the first, this means that the body has increased its stock of nitrogenous substances—proteids. If, on the contrary, the urine and fæces contain more nitrogen than the food taken at the same time, this denotes that the body is giving up part of its nitrogen—that is, a part of its own proteids has been decomposed. We can, from the quantity of nitrogen, as above stated, calculate the corresponding quantity of proteids by mutiplying by 6.25. Usually, according to VOIT'S proposition, the nitrogen of the urine is not calculated as decomposed proteids, but as decomposed muscle-substance or flesh. Lean meat contains on an average about 3.4% nitrogen; hence each gramme of nitrogen of the urine corresponds in round numbers to about 30 grms. flesh. The assumption that lean meat contains 3.4% nitrogen is arbitrary, as specially shown by PFLÜGER;[1] and the relationship of N : C in the proteids of dried meat, which is of great importance in certain experiments on metabolism, is given different by various experimenters, namely, 1 : 3.22 — 1 : 3.68. ARGUTINSKY[2] found in ox-flesh, after complete removal of fat and subtraction of glycogen, that the relationship was 1 : 3.24.

A disproportionally large part of the carbon leaves the body as carbon dioxide, which escapes chiefly through the lungs and skin. The remainder of the carbon is eliminated in the form of organic combinations by the urine and fæces, in which the quantity of carbon can be determined by elementary analysis. The quantity of gaseous carbon dioxide eliminated may be determined by means of PETTENKOFER'S respiration apparatus, or by other methods as described in the preceding chapter. By multiplying the quantity of carbon dioxide found by 0.273 we obtain the quantity of carbon eliminated as CO_2. If we compare the total quantity of carbon eliminated in various ways with the carbon contained in the food we obtain some idea as to the transformation of the carbon compounds. If the quantity of carbon in the food is greater than in the excreta, then the excess is deposited; while if the reverse be the case it shows a corresponding loss of bodily substance.

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[1] Pflüger's Arch., Bd. 51, S. 229.

[2] *Ibid.*, Bd. 55.

The nature of the substances here deposited or lost, whether they consist of proteids, fats, or carbohydrates, is learned from the total quantity of nitrogen of the excretions. The corresponding quantity of proteids may be calculated from the quantity of nitrogen, and, as the average quantity of carbon in the proteids is known, the quantity of carbon which corresponds to the decomposed proteids may be easily ascertained. If the quantity of carbon thus found is smaller than the quantity of the total carbon in the excreta it is then obvious that some other nitrogen-free substance has been consumed besides the proteids. If the quantity of carbon in the proteids is considered in round numbers as 53%,[1] then the relation between carbon (53) and nitrogen (16) is as 3.3 : 1. If we multiply the total quantity of nitrogen eliminated by 3.3 the excess of carbon in the eliminations over the product found represents the carbon of the decomposed non-nitrogenous compounds. For instance, in the case of a person experimented upon, 10 grms. nitrogen and 200 grms. carbon were eliminated in the course of 24 hours; then these 62.5 grms. proteid correspond to 33 grms. carbon, and the difference, $200 - (3.3 \times 10) = 167$, represents the quantity of carbon in the decomposed non-nitrogenous compounds. If we start from the simplest case, starvation, where the body lives at the expense of its own substance, then, since the quantity of carbohydrates as compared to the fats of the body is extremely small, in such cases in order to avoid mistakes the assumption must be made that the person experimented upon has only used fat and proteids. As animal fat contains on an average 76.5% carbon, the quantity of transformed fat may be calculated by multiplying the carbon by $\frac{100}{76.6} = 1.3$. In the case of the above example the person experimented upon would have used 62.5 grms. proteids and $167 \times 1.3 = 217$ grms. fat of his own body in the course of the 24 hours.

Starting from the nitrogen balance, we can calculate in the same way whether an excess of carbon in the food as compared with the quantity of carbon in the excreta is retained by the body as proteids or fat or as both. On the other hand, with an excess of carbon in the excreta we can calculate how much of the loss of the substance of the body is due to a consumption of the proteids or of fat or of both.

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[1] This figure is perhaps a little too high.

The quantity of water and mineral bodies voided with the urine and fæces can easily be determined. The quantity of water eliminated by the skin and lungs may be directly determined by means of PETTENKOFER's apparatus. The quantity of oxygen taken up is calculated as the difference between the weight of the individual before the experiment plus all the directly determined substances taken in, and the final weight of the individual plus all his excreta.

The oxygen may, according to the methods given in the preceding chapter, be directly determined, and such a determination with the simultaneous estimation of the carbon dioxide eliminated is of great importance in the study of metabolism.

On comparing the inspired and the expired air we learn, on measuring them when dry and at the same temperature and pressure, that the volume of the expired air is less than that of the inspired air. This depends upon the fact that not all of the oxygen appears again in the expired air as carbon dioxide, because it is not only used in the oxidation of carbon, but also in part in the formation of water, sulphuric acid, and other bodies. The volume of expired carbon dioxide is regularly less than the volume of the inspired oxygen, and the relation $\frac{CO^2}{O}$, which is called the *respiratory quotient*, is generally less than 1.

The magnitude of the respiratory quotient is dependent upon the kind of substances destroyed in the body. In the combustion of pure carbon one volume of oxygen yields one volume of carbon dioxide, and the quotient is therefore equal to 1. The same is true in the burning of carbohydrates, and in the exclusive decomposition of carbohydrates in the animal body the respiratory quotient must be approximately 1. In exclusive metabolism of proteids it is 0.73, and with the decomposition of fat it is 0.7. In starvation, as the animal draws on its own flesh and fat, the respiratory quotient must be a close approach to the latter figure. The respiratory quotient therefore gives important explanations on the quality of the material decomposed in the body, naturally with the supposition that the elimination of carbon dioxide, independent of the formation of carbon dioxide, is not influenced by special conditions, such as alternation of the respiratory mechanism.

It is also possible in systematized experimentation so to carry on the metabolism experiments that the decomposition material of the body—as shown by the respiratory quotient—remains qualitatively the same, at least for a short time. In such experiments it has been shown, especially by ZUNTZ[1] and his pupils, that the extent of oxygen consumption may be taken as a measure for the action of different influences on the extent of metabolism. This possibility is based on the fact proven by PFLÜGER[2] and his pupils, and by VOIT,[3] that the consumption of oxygen within wide limits is independent of the supply of oxygen, and is exclusively dependent upon the oxygen demand of the tissues. For certain reasons[4] the consumption of oxygen gives indeed a better conclusion than the elimination of carbon dioxide as to the extent of exchange of material and energy; but as the same quantity of oxygen (100 grms.) consumes different quantities of fat, carbohydrates, and proteids in the body—namely, 35, 84.4, and 74.4 grms. respectively—the respiratory quotient must also be determined, in order to ascertain the nature of the substance burnt in the body, by the simultaneous determination of the elimination of carbon dioxide.

I. Potential Energy and the Relative Nutritive Value of Various Organic Foods.

With the organic foods the organism receives a supply of potential energy which is converted into living force in the body. This potential energy of the various foods may be represented by the amount of heat which is set free in their combustion. This quantity of heat is expressed as calories, and a small calorie is the quantity of heat necessary to warm 1 grm. water from 0° to 1° C. A large calorie is the quantity of heat necessary to warm 1 kilo water 1° C. Here and in the following pages large calories are to be understood. We have numerous investigations by different experimenters, such as FRANKLAND, DANILEWSKI, RUBNER,[5] BERTHELOT,[6] STOH-

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[1] See Chapter XVII, foot-note 4, p. 604.

[2] Pflüger's Arch., Bdd. 6, 10 u. 14; Finkler, *ibid.*, Bd. 10; Finkler and Oertmann, *ibid.*, Bd. 14.

[3] Zeitschr. f. Biologie, Bdd. 11 u. 14.

[4] See Ad. Magnus-Levy, Pflüger's Arch., Bd. 55, S. 7.

[5] Zeitschr. f. Biologie, Bd. 21, where the works of Frankland and Danilewski are cited.

[6] Compt. rend., Tomes 102, 104, 110.

MANN,[1] and others, on the calorific value of different foods. The following results, which represent the calorific value of a few nutritive bodies on complete combustion outside of the body to the highest oxidation products, are taken from STOHMANN'S[2] latest work.

	Calories.
Casein	5.86
Ovalbumin	5.74
Conglutin	5 48
Proteid (average)	5.71
Animal tissue-fat	9.50
Butter-fat	9.23
Cane-sugar	3.96
Lactose	3.95
Dextrose	3.74
Starch	4.19

Fat and carbohydrates are completely burnt in the body, and we can therefore consider their combustion equivalent as a measure of the living force developed by them within the organism. We generally designate 9.3 and 4.1 calories for each grm. of substance as the average for the physiological calorific value of fats and carbohydrates respectively.

The proteids act differently from the fats and carbohydrates. They are only incompletely burnt, and they yield certain decomposition products, which, leaving the body with the excreta, still represent a certain quantity of potential energy which is lost for the body. The heat of combustion of the proteids is smaller within the organism than outside of it, and they must therefore be specially determined. For this purpose RUBNER[3] fed a dog on washed meat, and he subtracted from the heat of combustion of the food the heat of combustion of the urine and fæces, which corresponded to the food taken plus the quantity of heat necessary for the swelling up of the proteids and the solution of the urea. RUBNER has also tried to determine the heat of combustion of the proteids (muscle-proteids) decomposed in the body of rabbits in starvation. According to these investigations, the physiological heat of combustion in calories for each gramme of substance is as follows:

1 grm. of the Dry Substance.	Calories.
Proteids from meat	4.4
Muscle	4.0
Proteids in starvation	3.8
Fat (average for various fats)	9.3
Carbohydrates (calculated average)	4.1

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[1] Zeitschr. f. Biologie, Bd. 31.

[2] L. c.

[3] Zeitschr. f. Biologie, Bd. 21.

The physiological combustion value of the various foods belonging to the same group is not quite the same. It is, for instance, 3.97 calories for a vegetable proteid, conglutin, and 4.42 calories for an animal proteid body, syntonin. According to RUBNER we may consider the normal heat value per 1 grm. of animal proteid as 4.23 calories, and of vegetable proteid as 3.96 calories. When a person on a mixed diet takes about 60% of the proteids from animal foods and about 40% from vegetable foods, we may consider the value of 1 grm. of the proteid of the food as about 4.1 calories. The physiological value of each of the three chief groups of organic foods, by their decomposition in the body, is in round numbers as follows:

	Calories.
1 grm. proteid	4.1
1 " fat	9.3
1 " carbohydrate	4.1

As will be shown, the fats and carbohydrates may decrease the metabolism of proteids in the body, while, on the other hand, the quantity of proteids in the body or in the food acts on the metabolism of fat in the body. In physiological combustion the various foods may replace one another to a certain extent, and it is therefore important to know in what proportion they can replace one another. The investigations made by RUBNER have taught that this, if it relates to the force and heat production in the animal body, is in proportions that correspond with the figures of the heat value of the same. This is apparent from the following table. In this we find the weight of the various foods equal to 100 grms. fat, a part determined from experiments on animals and a part calculated from figures of the heat values.

TABLE 1.

100 grms. fat are equal to or isodynamic with:

	From Experiments on Animals.	From the Heat Value.	Difference, per cent.
Syntonin	225	213	+ 5.6
Muscle-flesh (dried)	243	235	+ 4.3
Starch	232	229	+ 1.3
Cane-sugar	234	235	− 0
Grape-sugar	256	253	− 0

From the given *isodynamic value* of the various foods it follows that these substances replace one another in the body almost in exact ratio to the potential energy contained in them. Thus in round numbers 227 grms. proteid and carbohydrate are equal to or isodynamic with 100 grms. fat in regard to source of energy, because each yields 930 calories on combustion in the body.

By means of recent very important calorimetric investigations RUBNER[1] has shown that the heat produced in an animal in several series of experiments extending over 45 days corresponded to within 0.47% with the physiological heat of combustion calculated from the decomposed body and foods.

This isodynamic law is of fundamental value in the study of metabolism and nutrition. By this law it is possible to consider the processes of metabolism as more uniform. The quantity of energy in the foods may be used as a measure for the total consumption of energy, and the knowledge of the quantity of energy in the foods must also be the basis for the calculation of dietaries for human beings under various conditions.

II. Metabolism in Starvation.

In starvation the decomposition in the body continues uninterruptedly, though with decreased intensity ; but, as it takes place at the expense of the substance of the body, it can only continue for a limited time. When an animal has lost a certain fraction of the mass of the body death is the result. This fraction varies with the condition of the body at the beginning of the starvation period. Fat animals succumb when the weight of the body has sunk to ½ of the original weight. Otherwise, according to CHOSSAT,[2] animals die as a rule when the weight of the body has sunk to ⅗ of the original weight. The period when death occurs from starvation not only varies with the varied nutritive condition at the beginning of starvation, but also with the more or less active exchange of material. This is more active in small and young animals than in large and older ones, but different classes of animals show an unequal activity. Children succumb in starvation in 3–5 days after having lost ¼ of their bodily mass. Grown persons, as observed on SUCCI,[3] may starve for 20 days without lasting injury ; and we have statements of even over 40–50 days' starvation. Dogs can live without food from 4–8 weeks, birds 5–20 days, snakes more than half a year, and frogs more than a year.

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[1] Zeitschr. f. Biologie, Bd. 30.

[2] Cited from Voit in Hermann's Handbuch, Bd. 6, Thl. 1, S. 100.

[3] See Luciani, Das Hungern. Hamburg u. Leipzig, 1890.

In starvation the *weight of the body* decreases. The loss of weight is greatest in the first few days, and then decreases rather uniformly. In small animals the absolute loss of weight per day is naturally smaller than in larger animals. The relative loss of weight—that is, the loss of weight of the unit of the weight of the body, namely, 1 kilo—is, on the contrary, greater in small animals than in larger ones. The reason for this is that the smaller animals have a greater surface of body in proportion to their mass than larger animals, and the greater loss of heat caused thereby must be replaced by a more active consumption of material.

It follows from the decrease in the weight of the body that the absolute extent of metabolism must diminish in starvation. If, on the contrary, we refer the extent of the metabolism to the unit of the weight of the body, namely, 1 kilo, we find that this quantity remains nearly unchanged during starvation. The investigations of ZUNTZ, LEHMANN, and others[1] on CETTI showed on the 3d to 6th day of starvation an average consumption 4.65 c. c. oxygen per kilo in one minute and on the 9th to 11th day an average of 4.73 c. c. The calories, as a measure of the metabolism, fell on the 1st to 5th day of starvation from 1850 to 1600 calories, or from 32.4 to 30 per kilo, and he remained nearly unchanged, if we refer to the unit of bodily weight.

As the metabolism in starvation takes place at the expense of the constituents of the body, it must take place in essentially the same way in both carnivora and herbivora. As the food of the herbivora is ordinarily richer in carbohydrates and non-nitrogenous nutritive bodies than that of the carnivora, so in starvation the body of the herbivora becomes relatively richer in proteids. On this account the elimination of nitrogen is increased in herbivora in the first part of the period of starvation. In carnivora the elimination of nitrogen decreases, as a rule, immediately at the beginning of the starvation, and in the later periods only small quantities of nitrogen are voided by herbivora as well as by carnivora.

The *extent of the metabolism of proteids,* or the elimination of nitrogen by the urine, which is a measure for the same, does not show in carnivora any uniform decrease during the entire period of starvation. During the first few days the elimination of nitrogen is greatest, and the quantity of the same depends essentially upon the amount of proteids in the organism and the nature of the food previously taken. The richer the body is in proteids from the food previously taken the greater is the metabolism of proteids, or, in other words, the elimination of nitrogen during the first days of starvation is greater. The rapidity with which the elimination of nitrogen decreases in the first days depends also, according to VOIT, upon the proteid condition of the body. It decreases more quickly—that is, the curve of the decrease is more sudden—the first days of starvation, as a rule, the richer the food was in proteids which was taken before starvation. This condition is apparent from the following table. This table contains three different starvation experiments made by VOIT[1] on the same dog. This dog received 2500 grms. flesh daily before the first series of experiments, 1500 grms. flesh daily before the second series, and a mixed food relatively poor in nitrogen before the third series.

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[1] Berlin. klin. Wochenschr., 1887.

TABLE II.

Day of Starvation.	Grammes of Urea Eliminated in the Twenty-four Hours.		
	Ser. I.	Ser. II.	Ser. III.
1	60.1	26.5	13.8
2	24.9	18.6	11.5
3	19.1	15.7	10.2
4	17.3	14.9	12.2
5	12.3	14.8	12.1
6	13.3	12.8	12.6
7	12.5	12.9	11.3
8	10.1	12.1	10.7

Other conditions, such as varying quantities of fat in the body, have an influence on the rapidity with which the nitrogen is eliminated during the first days of starvation. After the first few days the elimination of nitrogen, as is seen in the above table, is more uniform, and as the starvation proceeds it decreases as a rule very slowly and uniformly. Cases also occur in which the elimination of nitrogen becomes constant in these stages, and towards the end, indeed, the elimination of nitrogen increases. This so-called premortal increase always occurs as soon as the adipose tissue in the body has sunk to a certain point, and it also depends on the fact that as soon as the fat is consumed a corresponding increase in the decomposition of proteids is necessary for the generation of heat as well as of other forms of living force.

Besides the proteids the fat occurring in the body is also decomposed in starvation. Since fat has a diminishing influence on the destruction of proteids (see further on), the elimination of nitrogen in starvation is less in fat than in lean individuals. For instance, only 9 grms. of urea were voided in twenty-four hours during the later stages of starvation by a well-nourished and fat person suffering from disease of the brain, while I. Munk found that 20–29 grms. urea were voided daily by Cetti,[1] who had been poorly fed.

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[1] Physiol. des Stoffwechsels, etc., in Hermann's Handbuch, Bd. 6, Thl. 1, S. 89.

Like the destruction of proteids during starvation, the *decomposition of fat* proceeds uninterruptedly. The decomposition of fat does not show so great and rapid a decrease in the first days of starvation as the destruction of proteids. Pettenkofer and Voit found, for instance, in a starving dog the following losses of proteids and fat from the body on different days of starvation:

Table III.

Day.	Loss of		Loss of	
	Flesh.	Calories.[2]	Fat.	Calories.
2	341	297.3	86	799.8
5	167	145.6	103	957.9
8	138	120.1	99	920.7

The consumption of fat on the second day, when the decomposition of proteids was considerable, was indeed less than in the following days. The reason for this was that the animal had previously been fed with abundant quantities of meat (2500 grms.). If the exchange is expressed as calories we find for the fifth and eighth days of starvation that 13.2% and 11.5% respectively of the total calories was covered by the decomposition of proteids and 86.8% and 85.5% by the decomposition of fat. Other observations on animals as well as man have led to a similar result, and we can assume that in starvation ordinarily the greatest part of the expenditure is replaced by the decomposition of fat and only a small part by the decomposition of proteids.

The investigations on the *exchange of gas* in starvation have shown, as previously mentioned, that the absolute extent of the same is diminished, but that, when the consumption of oxygen and elimination of carbon dioxide is calculated on the unit of weight of the body, 1 kilo, this quantity quickly sinks to a minimum and then remains unchanged, or on the continuation of the starvation may indeed rise. It is a generally known fact that the body temperature of starving animals remains rather constant without showing any appreciable decrease during the greater part of the starvation period. The temperature of the animal first sinks a few days before death and starvation death occurs at about 33–30° C.

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[1] L. c.

[2] The calories of the decomposed proteids were calculated by the author, assuming that the flesh contains 3.4% nitrogen as proteids.

From what has been said on the respiratory quotient it follows that in starvation it is about the same as with exclusive fat and meat as food, namely, about 0.7. This is often the case, but it may also be indeed lower, 0.65—0.50, as observed in the cases of CETTI and SUCCI. As explanation for this unexpected behavior we admit of a storage of incompletely oxidized substances in the body during starvation.

Water passes uninterruptedly from the body in starvation even when none is given. If the quantity of water in the tissues rich in proteids is considered as 70–80%, and the quantity of proteids in the same 20%, then for each gramme of destroyed proteids about 4 grammes of water are set free. A special increase in the demand for water does not seem to occur in starving animals.

The *mineral svbstances* leave the body uninterruptedly in starvation until death, and the influence of the destruction of tissues is plainly perceptible by their elimination. Because of the destruction of tissues rich in potassium the proportion between potassium and sodium in the urine changes in starvation, so that, contrary to the normal conditions, the potassium is eliminated in proportionally greater quantities. MUNK also observed in CETTI's[1] case a relative increase in the phosphoric acid and calcium in the urine during starvation, which was due to an increased exchange of bone-substance.

TABLE IV.

	Pigeon (CHOSSAT).	Male Cat (VOIT).
Adipose tissue	93 per cent.	97 per cent.
Spleen	71 "	67 "
Pancreas	64 "	17 "
Liver	52 "	54 "
Heart	45 "	3 "
Intestine	42 "	18 "
Muscles	42 "	31 "
Testicles		40 "
Skin	33 "	21 "
Kidneys	32 "	26 "
Lungs	22 "	18 "
Bones	17 "	14 "
Nervous system	2 "	3 "

The question as to the participation of the different organs in the loss of weight of the body during starvation is of special interest. To illustrate this question we have given above the results of CHOS-

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[1] L. c.

SAT'S[1] experiments on pigeons and those of VOIT on a male cat. The results are percentages of weight lost from the original weight of the organ.

The total quantity of blood, as well as the quantity of solids contained therein, decreases, as PANUM[2] has shown, in the same proportion as the weight of the body. The statements in regard to the loss of water by different organs are somewhat contradictory; according to LUKJANOW,[3] it seems that the various organs act somewhat differently in this respect.

The above-tabulated results cannot serve as a measure of the metabolism in the various organs during starvation. For instance, the nervous system shows only a small loss of weight as compared with the other organs, but from this it must not be concluded that the exchange of material in this system of organs is least active. The condition may be quite different; for one organ may derive its nutriment during starvation from some other organ and exist at its expense. A positive conclusion cannot be drawn in regard to the activity of the metabolism in an organ from the loss of weight of that organ in starvation.

The knowledge of metabolism during starvation is of the greatest importance in the study of nutrition, and it forms to a certain extent the starting-point for the study of metabolism under different physiological and pathological conditions. To answer the question whether the metabolism of a person in a special case is abnormally increased or diminished it is naturally very important to know the average extent of metabolism of a healthy person under the same circumstances for comparison. This quantity can be called the abstinent value, namely, the extent of metabolism used in absolute bodily rest and inactivity of the intestinal tract. As measure of this quantity we determine according to GEPPERT-ZUNTZ the extent of gaseous exchange, and especially the consumption of oxygen, of a person lying down, best sleeping, in the early morning and at least 12 hours after a light meal not rich in carbohydrates. The gas volume reduced to 0° C. and 760 mm. Hg pressure is calculated on 1 kilo of body weight and for 1 minute. The results vary between 3 and 4.5 for the consumption of oxygen and between 2.5 and 3.5

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[1] Cited from Voit in Hermann's Handbuch, Bd. 6, Thl. 1, S. 96 u. 97.

[2] Virchow's Arch., Bd. 29.

[3] Zeitschr. f. physiol. Chem., Bd. 13.

c. c. for the carbon dioxide. As average we can accept 3.81 c. c. oxygen and 3.08 c. c. carbon dioxide.[1]

The extent of proteid destruction cannot be determined in transient experiments, and for these reasons only the values found after several days of starvation are useful. In the starvation experiments on CETTI and SUCCI the elimination of nitrogen per kilo in the fifth to the tenth starvation day was 0.150–0.202 grm. N.

III. Metabolism with Inadequate Nutrition.

The food may be quantitatively insufficient, and the final result is absolute inanition. The food may also be qualitatively insufficient or, as we say, inadequate. This occurs when any of the necessary nutritive bodies are absent in the food, while the others occur in sufficient or perhaps indeed in excessive amounts.

Lack of *Water* in the Food. The quantity of water in the organism is greatest during fœtal life, and then decreases with increasing age. Naturally, the quantity differs in various organs. The tissue in the body being poorest in water is the enamel, which is almost free, containing only 2 p. m. water, the teeth about 100 p. m., the fatty tissues 60–120 p. m. The bones with 140–440 p. m. and the cartilage with 540–740 p. m. are somewhat richer in water, while the muscles, blood, and glands with 750 to more than 800 p. m. are still richer. The quantity of water is even greater in the animal fluids (see preceding chapter), and the adult body contains in all about 630 p. m. water.[2] If we bear in mind that two thirds of the animal organism consists of water; that water is of the very greatest importance in the normal, physical composition of the tissues; moreover that all flow of juices, all exchange of substance, all supply of nutrition, all increase or destruction, and all discharge of the products of destruction are dependent upon the presence of water; besides this, that by its evaporation it is an important regulator of the temperature of the body,—we perceive that water must be necessary for life. If the loss of water be not replaced by fresh supplies sooner or later, the organism succumbs.

Lack of *Mineral Substances* in the Food. We are chiefly indebted to LIEBIG for showing that the mineral substances are just as necessary for the normal composition of the tissues and organs, and for the normal course of the processes of life, as the organic constituents of the body. The importance of the mineral constituents is evident from the fact that there is no animal tissue or animal fluid which does not contain mineral substance, and also from the fact that certain tissues or elements of tissues contain regularly certain mineral substances and not others, which explains the unequal division of the potassium and sodium compounds in the tissues and fluids. With the exception of the skeleton, which contains about 220 p. m. mineral bodies (VOLKMANN[1]), the animal fluids or tissues are poor in inorganic constituents, and the quantity of such only amounts, as a rule, to about 10 p. m. Of the total quantity of mineral substances in the organism, the greatest part occurs in the skeleton, 830 p. m., and the next greatest in the muscles, about 100 p. m. (VOLKMANN).

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[1] These figures are taken from v. Noorden's Lehrbuch der Path. des Stoffwechsels, S. 94.

[2] See Voit in Hermann's Handbuch, Bd. 6, Thl. 1, S. 345.

The mineral bodies seem to be partly dissolved in the fluids and partly combined with organic substances. In accordance with this the organism persistently retains, with food poor in salts, a part of the mineral substances, also such as are soluble, as the chlorides. On the burning of the organic substances the mineral bodies combined therewith are set free and may be eliminated. It is also admitted that they in part combine with the new products of the burning, and also that they in part are attached to organic nutritive bodies poor in salts or nearly salt-free, which are absorbed from the intestinal canal and are thus retained (VOIT, FORSTER[2]).

If this statement be correct, it is possible that a constant supply of mineral substances with the food is not absolutely necessary, and that the amount of inorganic bodies which must be administered is insignificant. The question whether this be so or not has not, especially in man, been sufficiently investigated; but generally we consider the need of mineral substances by man as very small. It may, however, be assumed that man usually takes with his food a considerable excess of mineral substances.

Investigations on animals in regard to the action of an insufficient supply of mineral substances with the food have been made by several investigators, especially FORSTER. He observed, on experimenting on dogs and pigeons with food as poor as possible in mineral substances, a very suggestive disturbance of the functions of the organs, especially the muscles and the nervous system, and death resulted after a time, indeed earlier than in complete starvation. In opposition to these observations BUNGE[1] has suggested that the early death in these cases was not caused by the lack of mineral salts, but more likely by the lack of bases necessary to neutralize the sulphuric acid formed in the burning of the proteids in the organism, which must be then taken from the tissues. In accordance with this view, BUNGE and LUNIN[2] also found on experimenting on mice that animals which received nearly ash-free food with the addition of sodium carbonate were kept alive twice as long as animals which had the same food without the addition of sodium carbonate. Special experiments also show that the carbonate cannot be replaced by an equivalent amount of sodium chloride, and that to all appearances it acts by combining with the acids formed in the body. The addition of alkali carbonate to the otherwise nearly ash-free food may indeed delay death, but cannot prevent it, and even in the presence of the necessary amount of bases death results for lack of mineral substances in the food.

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[1] See Voit in Hermann's Handbuch, Bd. 6, Thl. 1, S. 353.

[2] Zeitschr. f. Biologie, Bd. 9. See also Voit in Hermann's Handbuch, Bd. 6, Thl. 1, S. 354.

In the above series of experiments made by BUNGE the food of the animal consisted of casein, milk-fat, and cane-sugar. While milk alone was an adequate and sufficient food for the animal, BUNGE found that the animal could not be kept alive longer by food consisting of the above constituents of milk and cane-sugar with the addition of all the mineral substances of milk, than with the food mentioned in the above experiments with the addition of alkali carbonate. The question whether this result is to be explained by the fact that the mineral bodies of milk are chemically combined with the organic constituents of the same and can be assimilated only in such combinations, or whether it depends on other conditions, BUNGE leaves undecided. These observations, however, show how difficult it is to draw positive conclusions from experiments made thus far with food poor in salts. Further investigations on this subject seem to be necessary.

With an insufficient supply of *chlorides* with the food the elimination of chlorine by the urine decreases constantly, and at last it may stop entirely while the tissues still persistently retain the chlorides. These last are, at least in part, combined in the body with the organic substances which retain them. The great importance of such a retention of chlorides by the tissues is apparent if we bear in mind that the NaCl is not only a solvent for certain albuminous bodies, or a material for the elaboration of the gastric juice, but that it is also of the greatest importance as a so-called indifferent salt for the preservation of the normal consistency and the physiological imbibition relation of the tissues.

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[1] Lehrbuch d. physiol. Chem., 1. Aufl., S. 103.

[2] *Ibid.*, and Zeitschr. f. physiol. Chem., Bd. 5.

If there be a lack of sodium as compared with potassium, also if there be an excess of potassium compounds in any other form than KCl, the potassium combinations are replaced in the organism by NaCl, so that new potassium and sodium compounds are produced which are voided with the urine. The organism becomes poorer in NaCl, which therefore must be taken in greater amounts from the outside (BUNGE). This occurs habitually in herbivora, and in man with vegetable food rich in potash. For human beings, and especially for the poorer classes of people who live chiefly on potatoes and foods rich in potash, common salt is, under these circumstances, not only a condiment, but a necessary addition to the food (BUNGE[1]).

Lack of *Alkali Carbonates* or *Bases* in the Food. The chemical processes in the organism are dependent upon the presence of alkaline-reacting tissue-fluids, whose alkaline reaction is due to alkali carbonates. The alkali carbonates are also of great importance not only as a solvent for certain proteid bodies and as constituents of certain secretions, such as the pancreatic and intestinal juices, but they are also a means of transportation of the carbon dioxide in the blood. It is therefore easy to understand that a decrease below a certain point in the quantity of alkali carbonate must endanger life. Such a decrease not only occurs with lack of bases in the food which accelerates death by a relatively too great production of acids by the burning of the proteids (see above: BUNGE and LUNIN), but it also occurs when an animal is given dilute mineral acids for a certain time. In herbivora the fixed alkalies of the tissues combine with the mineral acids, and the animal succumbs after a time. In carnivora (and in man) the bases of the tissues are obstinately retained; the mineral acids unite with the ammonia produced by the decomposition of the proteids or their cleavage products, and carnivora can therefore be kept alive for a longer time.

Lack of *Earthy Phosphates*. With the exception of the impor-

[1] Zeitschr. f. Biologie, Bd. 9.

tance of the alkaline earths as carbonates and principally as phosphates in the physical composition of certain structures, such as the bones and teeth, their physiological importance is nearly unknown. The occurrence of earthy phosphates in all proteids, and the great importance of the earthy phosphates in the passage of the proteids from a soluble to a coagulable and solid state, make it probable that the earthy phosphates play an important part in the organization of the proteids. The action which an insufficient supply of alkali-earths with the food causes is connected with the interesting question as to the effect of this lack upon the bony structure. This action, as well as the various results obtained by experiments on young and old animals, has already been spoken of in Chap. X, to which we refer the reader.

Lack of *Iron*. As iron is an integral constituent of hæmoglobin, indispensable for the introduction of oxygen, so iron is an indispensable constituent of the food. In iron starvation iron is continually eliminated, even though in diminished amounts (DIETL,[1] v. HÖSLIN,[2] and others). From the observations of v. HÖSLIN on dogs it seems that an inadequate supply of iron with the food causes an insufficient formation of hæmoglobin. A special result of the lack of iron is chlorosis, which the physician has often to contend with and whose origin is not really a lack of iron in the food, but more likely an incomplete assimilation and absorption of the foods containing iron (BUNGE). The iron-salts as such seem not to be absorbed at all in the intestinal canal, or only to a very small extent, so that it is questionable whether their absorption has any importance worth noting. It seems more probable that the absorption of iron from the food takes place in the form of protein bodies (nucleoalbumin) containing iron (BUNGE); and the importance of the iron-salts in preventing the lack of hæmoglobin consists chiefly, according to BUNGE,[3] in that these salts counteract the decomposition in the intestine of the protein bodies containing iron, with a splitting off of iron as iron sulphide.

In the absence of *proteid bodies* in the food the organism must nourish itself by its own proteid substances, and on such nutrition it must earlier or later succumb. By the exclusive administration of fat and carbohydrates the consumption of proteids in these cases is reduced, for by an exclusive fat and carbohydrate diet the metabolism of proteids may indeed be smaller than in complete starvation (HIRSCHFELD,[1] KUMAGAWA,[2] KLEMPERER,[3] MUNK,[4] ROSENHEIM,[5] and others). In conformity with this the animal may be kept alive longer by food containing only non-nitrogenous bodies than in complete starvation.

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[1] Wien. Sitzungsber., Bd. 71, Abth. 3, 1875.

[2] Zeitschr. f. Biologie, Bd. 18.

[3] Zeitschr. f. physiol. Chem., Bd. 9.

The absence of *fats* and *carbohydrates* in the food affect carnivora and herbivora somewhat differently. It is unknown whether carnivora can be kept alive for any length of time by food entirely free from fat and carbohydrates. But it has been positively demonstrated that they can be kept alive a long time by feeding exclusively with meat freed as much as possible from visible fat (PFLÜGER[6]). Human beings and herbivora, on the contrary, cannot live for any length of time on such food. On one side they lose the property of digesting and assimilating the necessarily large amounts of meat, and on the other a distaste for large quantities of meat or proteids soon appears.

IV. Metabolism with Various Foods.

For the carnivora, as above stated, meat as poor as possible in fat may be a complete and sufficient food. As the proteids moreover take a special place among the organic nutritive bodies by the quantity of nitrogen they contain, it is proper that we first describe the exchange of material with an exclusively meat diet.

Metabolism with food rich in proteids, or feeding only with meat as poor in fat as possible.

By an increased supply of proteids the metabolization of proteids and the elimination of nitrogen is increased, and this in proportion to the supply of proteids.

If a certain quantity of meat has been given as food daily to carnivora and the quantity is suddenly increased, an increased metabolism of proteids or an increase in the quantity of nitrogen eliminated is the result. If we feed the animal daily for a certain time with larger quantities of the same meat, we find that a part of the proteids accumulates in the body, but this part decreases from day to day, while there is a corresponding daily increase in the elimination of nitrogen. In this way a nitrogenous equilibrium is established, that is, the total quantity of nitrogen eliminated is equal to the quantity of nitrogen in the absorbed proteids or meat. If, on the contrary, an animal which is in nitrogenous equilibrium, having been fed on large quantities of meat, is suddenly fed with a small quantity of meat per day, then the animal gives up its own bodily proteids, the amount decreasing from day to day. The elimination of nitrogen and the metabolism of proteids decrease constantly, and the animal may in this case also pass into nitrogenous equilibrium or nearly into this condition. These relations are illustrated by the following table (VOIT[1]):

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[1] Virchow's Arch., Bd. 114.

[2] *Ibid.*, Bd. 116.

[3] Zeitschr. f. klin. Med., Bd. 16.

[4] Du Bois-Reymond's Arch., 1891.

[5] *Ibid.*, S. 341 and Pflüger's Arch., Bd. 54.

[6] Pflüger's Arch., Bd. 50.

TABLE V.

	Grms. of Meat in the Food per Day.	
	Before the Test.	During the Test.
1.	500	1500
2.	1500	1000

Grms. of Flesh metabolized in Body per Day.						
1	2	3	4	5	6	7
1222	1310	1390	1410	1440	1450	1500
1153	1086	1088	1080	1027		

In the first case (1) the metabolism of flesh before the beginning of the actual experiment on feeding with 500 grms. meat was 447 grms., and it increased considerably on the first day of the experiment, after feeding on 1500 grms. meat. In the second case (2), in which the animal was previously in nitrogenous equilibrium with 1500 grms. meat, the metabolism of flesh on the first day of the experiment, with only 1000 grms. meat, decreased considerably, and on the fifth day a nearly nitrogenous equilibrium was obtained. During this time the animal gave up daily some of its own proteids. Between that point below which the animal loses from its own weight and the maximum, which seems to be dependent upon the digestive and assimilative capacity of the intestinal canal, a carnivora may be kept in nitrogenous equilibrium with varying quantities of proteids in the food.

The supply of proteids, as well as the proteid condition of the body, affects the extent of the proteid metabolism. A body which has become rich in proteids by a previous abundant meat diet must, to prevent a loss of proteids, take up more proteid with the food than a body poor in proteids.

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[1] Hermann's Handbuch, Bd. 6, Thl. 1, S. 110.

PETTENKOFER and VOIT have made investigations on the *metabolism of fat* with an exclusively albuminous diet. These investigations have shown that by increasing the quantity of proteids in the food the daily metabolism of fat decreases, and they have drawn the conclusion from these experiments, as detailed in Chapter X, that even a formation of fat may take place under these circumstances. The objections presented by PFLÜGER against these experiments are also mentioned in this chapter, and KUMAGAWA[1] has recently published a new and important investigation on this subject.

KUMAGAWA caused two dogs of the same litter to fast for over 20 days in order to remove the body fat. One of the dogs (the control dog) was then killed and the total fat determined. The other animal received meat poor in fat (with a known quantity of ether extractives, glycogen, nitrogen, water, and ash) in as large quantities as it could endure, and this feeding with meat was continued (about 50 days) until a marked increase in bodily weight had taken place. The quantity of nitrogen in the urine and fæces during this period was also determined, and finally the animal was killed and the total quantity of fat determined. The results were that the fat formed during the period of feeding corresponded exactly with the quantity existing in the meat fed to the animal and formed from the glycogen of the meat. In this case no fat formation from proteid was found, and according to KUMAGAWA the animal body under normal circumstances has no ability of forming fat from proteid.

According to PFLÜGER's doctrine, which has received support from these investigations, the proteid can influence the formation of fat only in an indirect way, namely, in that it is consumed instead of the non-nitrogenous bodies and hence the fat and fat-forming carbohydrates are spared. If sufficient proteid is introduced in the food to satisfy the total nutritive requirements, then the decomposition of fat stops; and if also non-nitrogenous food is taken at the same time, this is not consumed, but is stored up in the animal body, the fats as such, and the carbohydrates at least in great part as fat.

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[1] Zur Frage der Fettbildung aus Eiweiss im Thierkörper. Mittheil. der med. Fakultät der kaiserl. Japan. Universität zu Tokio, Bd. 3, No. 1, 1894.

PFLÜGER calls the "nutritive requirement" as the smallest quantity of lean meat which produces nitrogenous equilibrium without causing any decomposition of fat or carbohydrates. At rest and at an average temperature it is found for dogs to be 2.073 grms. nitrogen (in meat fed) per kilo of flesh weight (not bodily weight, as the fat, which often forms a considerable fraction of the weight of the body, cannot as it were be used as dead measure). Even when the supply of proteid is in excess of the nutritive requirements, PFLÜGER has found that the proteid metabolism increases with an increased supply until the limit of digestive power is reached, which limit is about 2600 grms. meat with a dog weighing 30 kilos. In these experiments of PFLÜGER's all of the excess of proteid introduced was not completely decomposed, but a part was retained by the body. PFLÜGER therefore defends the proposition "that an exclusive proteid supply, without fat or carbohydrate, does not exclude a proteid fattening."

From what has been said on proteid metabolism in starvation and with one-sided proteid food it follows that the proteid metabolism in the animal body never stops, that the extent is dependent in the first place upon the extent of proteid supply, and that the animal body has the property, within wide limits, of accommodating the proteid metabolism to the proteid supply.

These and certain other peculiarities of proteid metabolism have led VOIT to the view that all proteids in the body are not decomposed with the same ease. VOIT differentiates the proteids fixed in the tissue-elements, so-called organized proteids, *tissue-proteids*, from those proteids which circulate with the fluids in the body and its tissues and which are taken up by the living cells of the tissues from the interstitial fluids washing them and destroyed. These *circulating proteids* are, according to VOIT, more easily and quickly destroyed than the tissue-proteids. When, therefore, in a fasting animal which has been previously fed with meat an abundant and quickly decreasing decomposition of proteids takes place, while in the further course of starvation this proteid metabolism becomes less and more uniform, this depends upon the fact that the supply of circulating proteids is destroyed chiefly in the first days of starvation and the tissue-proteids in the last days.

The tissue-elements constitute an apparatus of a relatively stable nature, which has the power of taking proteids from the fluids washing the tissues and digesting them, while a few proteids, the tissue-proteids, are ordinarily disorganized to only a small extent, about 1% daily (VOIT). By an increased supply of proteids the activity of the cells and their ability to decompose nutritive proteids is also increased to a certain degree. When nitrogenous equilibrium is obtained after increased supply of proteids, it denotes that the decomposing power of the cells for proteids has increased so that the same quantity of proteids is metabolized as is supplied to the body. If the proteid metabolism is decreased by the simultaneous administration of other non-nitrogenous foods (see below), a part of the circulating proteids may have time to become fixed and organized by the tissues, and in this way the mass of the flesh of the body increases. During starvation or with lack of proteids in the food the reverse takes place, for a part of the tissue proteids is converted into circulating proteids which are metabolized, and in this case the flesh of the body decreases.

VOIT's doctrine has been severely attacked by PFLÜGER.[1] PFLÜGER states, basing his statement on an investigation made by one of his pupils, SCHÖNDORFF,[2] that the extent of proteid destruction is not dependent upon the quantity of circulating proteids, but upon the nutritive condition of the cells for the time being—a view which is not very contradictory of VOIT's doctrine, if the AUTHOR does not misunderstand PFLÜGER's statement. VOIT[3] has, as is known, stated that the conditions of the destruction of substances in the body exist in the cells, and also that the circulating proteid, likewise according to VOIT, is first metabolized after having been taken up by the cells from the fluids washing them. The organized proteid, which is fixed by the cells and has become a part of the same, is destroyed less readily, according to VOIT, than the proteid taken up by the cells from the nutritive fluid, which serves as material for the chemical construction of the very much more complicated organized proteids. This nutritive proteid, which circulates with the fluids before it is taken up by the cells, and which can exist in store in the cells as well as in the fluids, which corresponds to VOIT's view, has been called circulating proteid or supply proteid by him. It is clear that these names may lead to misunderstanding, and therefore too much stress should not be put on them. The most essential part of VOIT's doctrine is the supposition that the food proteid of the cells is more easily destroyed than the organized, real protoplasmic proteid, and this statement can hardly, for the present, be considered as refuted or exactly proven.

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[1] Pflüger's Arch., Bd. 54.

[2] *Ibid.*, Bd. 54.

[3] Zeitschr. f. Biologie, Bd. 11.

This question is intimately connected with another, namely, whether the food proteids taken up by the cells are metabolized as such or whether they are first organized. The investigations of Panum[1] and Falck[2] on the transitory progress of the elimination of urea after a meal rich in proteids throws light on this question. From the investigations on a dog it was found that the elimination of urea increases almost immediately after a meal rich in proteids, and that it reaches its maximum in about six hours, when about one half of the quantity of nitrogen corresponding to the administered proteids is eliminated. If we also recollect that, according to an observation of Schmidt-Mülheim[3] on a dog, about 37% of the given proteids are absorbed in the first two hours after the meal and about 59% in the course of the first six hours, we may then infer that the increased elimination of nitrogen after a meal is due to a metabolization of the digested and assimilated proteids of the food not previously organized. If we admit that the metabolized proteid must have been organized, then the greatly increased elimination of nitrogen after a meal rich in proteids supposes a far more rapid and comprehensive destruction and reconstruction of the tissues than has been generally admitted and not proven.

It has been stated above that other foods may decrease the metabolism of proteids. Gelatin is such a food. *Gelatin* and the *gelatin-formers* do not seem to be converted into proteid in the body, and this last cannot be entirely replaced by gelatin in the food. For example, if a dog is fed on gelatin and fat, its body sustains a loss of proteids even when the quantity of gelatin is so large that the animal, with an amount of fat and meat containing just the same quantity of nitrogen as the gelatin in question, may remain in nitrogenous equilibrium. On the other hand, gelatin, as Voit,[4] Panum and Oerum[5] have shown, has a great value as a means of sparing the proteids, and it may decrease the metabolism of proteids to a still greater extent than fats and carbohydrates.

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[1] Nord. med. Arkiv., Bd. 6.

[2] Cited from Voit in Hermann's Handbuch, Bd. 6., Thl. 1, S. 107.

[3] Du Bois-Reymond's Arch., 1879.

[4] L. c., S. 123.

[5] Nord. med. Arkiv., Bd. 11

This is apparent from the following summary of VOIT's experiments on a dog:

TABLE VI.

Food per Day.				Flesh.	
Meat.	Gelatin.	Fat.	Sugar.	Metabolized.	On the Body.
400	0	200	0	450	− 50
400	0	0	250	439	− 39
400	200	0	0	256	+ 44

I. MUNK[1] has later arrived at similar results by means of more decisive experiments. He found in dogs that on a mixed diet which contained 3.7 grms. proteid per kilo of body, of which hardly 3.6 grms. was metabolized, nearly ⅚ could be replaced by gelatin. The same dog metabolized on the second starvation day three times as much proteid as with the gelatin feeding. MUNK states also that gelatin has a much greater sparing action on proteids than the fat or the carbohydrates.

This ability of gelatin to spare the proteids is explained by VOIT by the statement that the gelatin is decomposed instead of a part of the circulating proteids, whereby a part of this last may be organized.

Gelatin may also decrease somewhat the consumption of fat, although it is of less value in this respect than the carbohydrates.

The question of nutritive value of *peptones* stands in close relation to the nutritive value of the proteids and gelatin. The early investigations made by MALY, PLOS'Z and GYERGYAY, and ADAMKIEWICZ[2] have led to the conclusion that an animal with food which contains no proteids besides peptones may not only preserve its nitrogenous equilibrium, but its proteid condition may even increase. According to recent, more exact investigations of POLLITZER, ZUNTZ,[3] and MUNK[3] the albumoses and peptones have the same nutritive value as proteids, at least in short experiments. According to POLLITZER this is true for different albumoses as well as for true peptone. Contrary to this view VOIT[4] is of the opinion that the albumoses and peptones can replace the proteids only for a short time, not indefinitely. According to VOIT the albumoses and peptones, like gelatin, may, by their ability to spare proteid, entirely or nearly arrest the consumption of proteid, but cannot pass into proteid.

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[1] Pflüger's Arch., Bd. 58.

[2] Cited from page 329.

[3] See Maly's Jahresber., Bd. 19, S. 352 u. 402.

[4] L. c., S. 394.

From experiments made by WEISKE[1] and others on herbivora it appears that *asparagin* may spare proteid in such animals. In carnivora (I. MUNK[2]) and in mice (VOIT and POLITIS[3]) it was found that asparagin does not seem to have any sparing action on the proteids,[4] or only a very slight action. It is not known how it acts in man.

Metabolism on a Diet consisting of Proteid, with Fat and Carbohydrate. Fat cannot arrest or prevent the *metabolism of proteids;* but it can decrease it, and so spare the proteids. This is apparent from the following table of VOIT.[5] A is the average for three days, and B for six days.

TABLE VII.

	Food.		Flesh.	
	Meat.	Fat.	Metabolized.	On the Body.
A..................	1500	0	1512	− 12
B..................	1500	150	1474	+ 24

According to VOIT the adipose tissue of the body acts like the food-fat, and the proteid-sparing effect of the former may be added to that of the latter, so that a body rich in fat may not only remain in nitrogenous equilibrium, but may even add to the store of bodily proteids, while in a lean body with the same food containing the same amount of proteids and fat there would be a loss of proteids. In a body rich in fat a greater quantity of proteids is protected from metabolism by a certain quantity of fat than in a lean body.

Because of the sparing action of fats an animal by the addition of fat to its food may, as is apparent from the tables, increase its proteid condition with a quantity of meat which is insufficient to preserve nitrogenous equilibrium.

Like the fats the carbohydrates have a sparing action on the proteids. By the addition of carbohydrates to the food the carnivor not only remains in nitrogenous equilibrium, but the same quantity of meat which in itself is insufficient and which without carbohydrates would cause a loss of weight in the body may with the addition of carbohydrates produce a deposit of proteids. This is apparent from the following table[1]:

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[1] Zeitschr. f. Biologie, Bdd. 15 u. 17 and Centralbl. f. d. med. Wissensch., 1890, S. 945.

[2] Virchow's Arch., Bdd. 94 u. 98.

[3] Zeitschr. f. Biologie, Bd. 28.

[4] See Manthner, *ibid.*, Bd. 28, and Gabriel, *ibid.*, Bd. 29, and Voit, *ibid.*, S. 125.

[5] See Voit in Hermann's Handbuch, Bd. 6, S. 130.

TABLE VIII.

Food.				Flesh.	
Meat.	**Fat.**	**Sugar.**	**Starch.**	**Metabolized.**	**On the Body.**
500	250	...	...	558	− 58
500	...	300	...	466	+ 84
500	...	200	. .	505	− 5
800	...	...	250	745	+ 55
800	200	...	...	773	+ 27
2000	...	...	200–300	1792	+208
2000	250	...	...	1883	+117

The sparing of proteid by carbohydrate is greater, as shown by the table, than by fats. According to VOIT the first is on an average 9% and the other 7% of the administered proteid, without a previous addition of non-nitrogenous bodies. Increasing quantities of carbohydrates in the food decrease the proteid metabolism more regularly and constantly than increasing quantities of fat.

The law as to the increased proteid metabolism with increased proteid supply applies also to food consisting of proteid with fat and carbohydrates. In these cases the body tries to adapt its proteid metabolism to the supply; and when the daily calorie supply is completely covered by the food, the organism can, within wide limits, be in nitrogenous equilibrium with different quantities of proteid.

The upper limit to the possible proteid metabolism per kilo and per day has only been determined for herbivora. It is not known for human beings, and its determination is from a practical standpoint of secondary importance. What is more important is to ascertain the lower limit, and on this subject we have several investigations on man as well as animals by HIRSCHFELD, KUMAGAWA, KLEMPERER, MUNK, ROSENHEIM,[2] and others. It follows from these investigations that the lower limit of proteid needed for human beings for a week or less is about 30–40 grms. proteid or 0.4–0.6 grm. per kilo with a body of average weight. v. NOORDEN[3] considers 0.6 grm. proteid (assimilated proteid) per kilo and per day as the lower limit. The above-mentioned figures are only valid for short series of experiments; still we have the observations of

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[1] Voit in Hermann's Handbuch, Bd. 6, S. 143.

[2] See foot-notes 1–5, page 630

[3] Grundriss einer Methodik der Stoffwechseluntersuchungen. Berlin, 1892

E. VOIT and CONSTANTINIDI[1] on the diet of a vegetarian in which the proteid condition was kept nearly but not completely maintained with about 0.6 grm. proteid per kilo.

According to VOIT's normal figures, which will be spoken of below for the nutritive need of man, an average working man of about 70 kilos weight on a mixed diet requires about 40 calories per kilo (two calories or net calories, namely, the combustion value of the assimilated foods). In the above experiments with food very poor in proteid the demand for calories was considerably greater, as for instance in certain cases it was 51 (KUMAGAWA) or even 78.5 calories (KLEMPERER). It therefore seems as if the above very low supply of proteid was only possible with great waste of non-nitrogenous food; but in opposition to this we must recall that in VOIT and CONSTANTINIDI's experiments on the vegetarian, who for years was used to a food very poor in proteid and rich in carbohydrate, the calories only amounted to 43.7 per kilo. It is an open question how a nitrogenous equilibrium can exist also on a diet very poor in nitrogen, when the need of calories is only just covered by the total supply.

In MUNK's and ROSENHEIM's experiments on dogs the food poor in proteids must have raised the total supply of calories considerably. These experiments also teach that in dogs the continuous administration for a long time of food poor in proteid has an action on the health of the animal and may even cause death. In the experiments recently published by ROSENHEIM, which extended over two months, 2 grms. proteid per kilo of body was not sufficient to keep the animal healthy although the heat value of the food taken up amounted to 110 calories per kilo.

The very important question as to the conditions for the deposition of fat and flesh on the body stands in close connection to what has just been said in regard to foods consisting of proteid and non-nitrogenous food-stuffs. In this connection we must recall in the first place that all fattening presupposes an overfeeding, i.e., a supply of food-stuffs which is greater than that metabolized at the same time.

In carnivora, as shown by the investigations of VOIT and PFLÜGER, a very inconsiderable metabolized proteid, in proportion to the deposition of flesh, may take place with exclusive meat food. In man and herbivora, on the contrary, the demand for calories may not be covered by proteid alone, and the question as to the conditions of fattening with a mixed diet is of importance.

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[1] C. Voit, Zeitschr. f. Biologie, Bd. 25.

These conditions have also been studied on carnivora, and here, as VOIT has shown, the relationship between proteid and fat (and carbohydrates) is of great importance. If considerable fat is given in proportion to the proteid of the food, as with average quantities of meat with considerable addition of fat, then nitrogenous equilibrium is only slowly attained and the daily deposit of flesh, though not large, but quite constant, may be considerable in the course of time. If, on the contrary, much meat besides proportionally little fat is given, then the deposit of proteid with increased metabolism is smaller day by day, and nitrogenous equilibrium is attained in a few days. In spite of the daily, somewhat larger deposit, the total flesh deposit is not considerable in these cases. The following experiment of VOIT may serve as example:

TABLE IX.

Number of Days of Experimentation.	Food.		Total Deposit of Flesh.	Daily Deposit of Flesh.	Nitrogenous Equilibrium
	Meat, grms.	Fat, grms.			
32	500	250	1792	56	not attained
7	1800	250	854	122	attained

The greatest absolute deposition of flesh in the body was obtained in these cases with only 500 grms. flesh and 250 grms. fat, and even after 32 days the nitrogenous equilibrium had not occurred. On feeding with 1800 grms. meat and 250 grms. fat the nitrogenous equilibrium occurred after 7 days; and though the deposition of flesh per day was greater, still the absolute deposit was not one half as great as in the former case. Inasmuch as the quantity of proteids does not decrease below a certain amount, it seems that the most abundant and most lasting deposition of flesh is obtained with a food which does not contain too much proteids in proportion to the fat. The same is also true of a diet consisting of proteids and carbohydrates.

The experiments of KRUG[1] on himself, under the direction of v. NOORDEN, give us information as to the practicability of flesh deposition in man. With abundant food (2590 cal. = 44 cal. per kilo) KRUG was close to nitrogenous equilibrium for six days. He then increased the nutritive supply to 4300 cal. = 71 cal. per kilo for 15 days by the addition of fat and carbohydrate, and in this time 309 grms. proteid, corresponding to 1455 grms. flesh, was spared. Of the excess of administered calories in this case only 5% was used for flesh deposit and 95% for fat deposit. As the large, excessive quantity of food was only transitorily and reluctantly eaten, this experiment, as v. NOORDEN has correctly emphasized, has placed the difficulty of flesh deposition in another light. We must admit with v. NOORDEN that it is impossible to produce a permanent flesh deposit in man by overfeeding, and that it is not possible to make a person muscle-strong by excessive feeding.

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[1] Cited from v. Noorden's Lehrbuch der Path. des Stoffwechsels. Berlin, 1893, S. 120.

Flesh deposition is, according to v. NOORDEN, a function of the specific development energy of the cells and the cell-work to a much higher extent than the excess of food. Therefore we observe, according to v. NOORDEN, abundant flesh deposition (1) in each growing body; (2) in those no longer growing but whose body is accustomed to increased work (hypertrophy of the muscles by work); (3) whenever, by previous insufficient food or by disease, the flesh condition of the body has been diminished and is compensated by abundant food. The deposition of flesh is in these cases an expression of the regenerative energy of the cells.

The experiences of cattle-raisers show that in food-animals a flesh deposit does not occur, or at least is only inconsiderable, on over-feeding. The individuality and the race of the animal is of importance for flesh deposition.

As a direct formation of fat is denied, and if it does occur it is only very insignificant, the most essential requisite for a fat deposition must be an overfeeding with non-nitrogenous nutritive bodies. The extent of fat deposition is determined by the excess of administered calories over those used. If a large part of the caloric demand is covered by proteid, then a greater part of the simultaneously given non-nitrogenous food-stuffs is spared, i.e., used for fat deposition. But as proteid and fat are expensive nutritive bodies as compared with carbohydrates, the supply of greater quantities of carbohydrates is important for fat deposition. The body decomposes less substance at rest than during activity. Bodily rest, besides a proper combination of the three chief groups of organic foods, is therefore also an essential requisite for an abundant fat deposit.

The fat formed in fat deposition originates, as stated above, entirely from the carbohydrates according to Pflüger's doctrine. In this fat-formation, as suggested by Hanriot[1] and Pflüger,[2] a splitting off of carbon dioxide takes place from the carbohydrates. This carbon dioxide, which in excessive feeding with carbohydrates is expired, has, according to Pflüger, a double origin. It is in part split off from the carbohydrates in the formation of fat, and it originates in part from the combustion of carbohydrates. This behavior explains the circumstance that after partaking large quantities of carbohydrates the respiratory quotient, as first shown by Hanriot and then also by M. Bleibtreu,[3] was raised under circumstances to 1.2–1.3.

Action of certain other Bodies on Metabolism. *Water.* If a quantity in excess of that which is necessary is introduced into the organism, the excess is quickly and principally eliminated with the urine. This increased elimination of urine causes in fasting animals (Voit,[4] Forster[5]), but not to any appreciable degree in animals taking food (Seegen,[6] Salkowski and Munk,[7] Mayer,[8] Dubelir[9]), an increased elimination of urea. The reason for this increased elimination is sought for in the fact that the abundant drinking of water causes a complete washing out of the urea from the tissues. Another view, which is defended by Voit, is that because of the more active current of fluids after taking large quantities of water an increased metabolism of proteids takes place. Voit considers this explanation the correct one, although he does not deny that by the abundant administration of water a more complete washing out of the urea from the tissues takes place.

In regard to the action of water on the formation of fat and its metabolism, the view that free drinking of water is favorable for the deposition of fat seems to be generally admitted, while taking only very little water acts against its formation.

Salts. The excretion of urine, even when no great quantities of water are taken, is increased by common salt, and the elimination of urea is also increased at the same time. The same two possibilities may be considered for this last as in the action of water on the excretion of urea. The experiments continued for a long time by Voit, in which the absolute increase of the elimination of urea was considerable (106 grms. in 49 days), render the conclusion probable that common salt somewhat increases the metabolism of the proteids. Dubelir has obtained contrary results which he considers was due to giving the animal large quantities of common salt. It is possible that the decomposition activity of the cells may be reduced on giving large quantities of salt. Certain other salts, such as potassium chloride, sodium sulphate, sodium phosphate, sodium acetate, saltpetre, and ammonium chloride, also seem to act like common salt. Sodium borate and the sodium salts of salicylic and benzoic acids also seem to have an increased action on the metabolism of proteids.

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[1] Compt. rend., Tome 114.

[2] Pflüger's Arch., Bd. 52, S. 45.

[3] *Ibid.*, Bd. 56.

[4] Untersuch. über den Einfluss des Kochsalzes, etc. München, 1860.

[5] Cited from Voit in Hermann's Handbuch, Bd. 6, S. 153.

[6] Wien. Sitzungsber., Bd. 63.

[7] Virchow's Arch., Bd. 71.

[8] Zeitschr f. klin. Med., Bd. 2.

[9] Zeitschr. f. Biologie, Bd. 28.

Alcohol. The question as to how far the alcohol absorbed in the intestinal canal is burnt in the body, or whether it leaves the body unchanged by various channels, has been the subject of much discussion. To all appearances the greatest part of the alcohol is burnt. According to Bodländer,[1] 1.18% of the alcohol taken is eliminated with the urine, 0.14% by the evaporation from the skin, and 1.6% with the expired air. The remainder, or about 97%, is burnt in the body. As the alcohol is in greatest part burnt in the body and has a high calorific value (1 grm. = 7 cal.), then the question arises whether it acts sparingly on other bodies, and whether it is to be considered as a nutritive body. The investigations made to decide this question have led to no decisive result. In the experiments on the elimination of nitrogen in human beings sometimes a diminished (Hammond, E. Smith, Obernier), sometimes an unchanged (Parkes and Wollowicz[2]), while in other cases an increased (Forster and Romeyn[3]) elimination of nitrogen was observed after the administration of small amounts of alcohol. In the recent experiments of Stammreich and v. Noorden[4] alcohol could only replace the isodynamic quantity of non-nitrogenous food-stuffs, without an essential influence on the proteid condition of the body, in a food richer in proteid than ordinarily. MIURA[1] could not find any sparing action on proteids by alcohol in his experiments, and according to him alcohol cannot replace the sparing action of carbohydrate on proteid. FOKKER[2] and I. MUNK[3] after the administration of small quantities of alcohol to dogs found a diminished, and after large quantities an increased, metabolism of proteids. CHITTENDEN, NORRIS, and E. SMITH[4] make the statement, based on their experiments with 1.9, 2.3, and 2.7 c. c. alcohol per kilo of dog per diem, that alcohol acts like a non-nitrogenous nutritive body in regard to its sparing action on proteids.

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[1] Pflüger's Arch., Bd. 32.

[2] In regard to the older investigations see Voit in Hermann's Handbuch, Bd. 6, S. 170.

[3] Maly's Jahresber., Bd. 17, S. 400.

[4] v. Noorden, Alkohol als Sparmittel. Berlin. klin. Wochenschr., 1891.

Many observations have been made on animals in regard to the extent of exchange of gas after taking alcohol. The results in these cases are somewhat different, depending upon the size of dose and the kind of animal. In an investigation on the human body ZUNTZ and BERDEZ,[5] and also GEPPERT,[6] observed no essential change in the respiratory exchange of gas after small, non-intoxicating doses of alcohol. As alcohol is in greatest part burnt up in the body and the exchange of gas is nevertheless not essentially raised, it seems as if the alcohol diminishes the combustion of other bodies and thereby has a sparing value. Corresponding to this, as is well known, a deposition of fat may take place in the body under the influence of alcohol. The nutritive value of alcohol may be of essential importance only in certain cases, as large quantities of alcohol taken at once or the continued use of smaller quantities has injurious action on the organism. Alcohol may therefore be considered as a nutritive body only in exceptional cases, and it otherwise must be considered as an article of luxury.

Coffee and *tea* have no positively proved action on the exchange of material, and their importance lies chiefly in their action upon the nervous system. It is impossible to enter into the action of various therapeutic agents upon metabolism.

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[1] Zeitschr. f. klin. Med., Bd. 20. Cited from Maly's Jahresber., Bd. 22, S. 461.

[2] Cited from Voit in Hermann's Handbuch, Bd. 6, S. 170.

[3] Du Bois-Reymond's Arch., 1879, S. 163.

[4] Journal of Physiology, Vol. 12

[5] See Maly's Jahresber., Bd. 7, S. 343.

[6] Arch. f. Path. u. Pharm., Bd. 22.

V. The Dependence of Metabolism on Other Conditions.

The previously mentioned so-called abstinence value, i.e., the extent of metabolism with absolute bodily rest and inactivity of the intestinal tract, serves best as a starting-point for the study of metabolism under various external circumstances. The metabolism going on under these conditions leads in the first place to the production of heat, and it is only to a subordinate degree dependent upon the work of the circulatory and respiratory apparatus and the activity of the glands. According to a calculation by ZUNTZ,[1] only 10–20% of the total calories of the abstinence value belongs to the circulation and respiration work.

The extent of the abstinence value depends in the first place upon the heat production necessary to cover the loss of heat, and this heat production is in turn dependent upon the relationship between the weight of body and the surface of the body.

Weight of Body and Age. The greater the mass of the body the greater the absolute consumption of material; while on the contrary, other things being equal, a small individual of the same species of animals metabolizes absolutely less, but relatively more as compared with the unit of the weight of the body. It must be remarked that we mean flesh weight when we say body weight. The extent of the metabolism is dependent upon the quantity of living cells, and a very fat individual therefore decomposes less substance per kilo than a lean person of the same weight of body. In women, who generally have less bodily weight and a greater quantity of fat than men, the metabolism in general is smaller, and the latter is ordinarily about ⅘ of that of men. Otherwise sex does not seem to have any special influence on the exchange of material.

The essential reason why small animals decompose relatively more substance, i.e., as calculated on the kilos of the body, than large ones is that the smaller animals have greater bodily surface in proportion to their mass. On this account the loss of heat is greater, which causes increased heat production, i.e., a more active metabolism. This is also the reason why young individuals of the same kind show a relatively greater decomposition than older ones. RUBNER,[2] whom we have to thank especially for our knowledge in regard to the bearing of the relative surfacial development on the extent of metabolism, has given us the following table on this point with respect to man:

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[1] Cited from v. Noorden's Lehrbuch, etc., S. 97.

[2] Zeitschr. f. Biologie, Bdd. 21 u. 19.

TABLE X.

	Calories in 24 Hours after Subtracting the Heat of Combustion of the Fæces.	Calories in 24 Hours per Kilo.	Surface in Square Centimetres.	Calories per Square Centimetre of Surface.
Children weighing 4.03 kilos..	368	91.3	3013	1221
" " 11.8 " ..	966	81.5	7191	1343
" " 16.4 " ..	1213	73.9	7681	1579
" " 23.7 " ..	1411	59.5	10156	1389
" " 30.9 " ..	1784	57.7	12122	1472
" " 40.4 " ..	2106	52.1	14491	1452
Man " 67.0 " ..	2843	42.4	20305	1399

If we exclude the smallest, actively growing children, in whose case special conditions govern, we find that the heat production for the unit of surface of body varies only a few per cent from the average of 1447 cal. We see how the relative extent of surface decreases with an increase in the mass of the body. Correspondent with this the metabolism per kilo of body weight also decreases, and it is smallest in adults.

A similar result was obtained by RICHET[1] in his investigations on the elimination of carbon dioxide in dogs of various sizes, as elucidated in the following table:

TABLE XI.

Average Weight of Body in Kilos.	CO_2 eliminated in Grammes per Kilo in 1 Hour.	Surface of Body in Square Centimetres.	CO_2 eliminated in Grammes per 1000 Square Centimetres.
24 0	1.026	9296	2 65
13.5	1.210	6272	2.60
11.5	1.380	5656	2 81
9 0	1.506	4816	2.81
6.5	1.624	3920	2.69
5.0	1.688	3282	2 57
3 1	1.964	2341	2.71
2.3	2.265	1926	2.70

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[1] Arch. de Physiol. (5), Bd. 2.

The raising of the metabolism which is necessary to cover the loss of heat because of the relatively larger surface of body in small animals is due, according to RICHET, to the influence of the nervous system, which may be reduced by chloral hydrate. In the last case the quantity of carbon dioxide produced per kilo in dogs of various sizes is nearly the same.

The question whether the active metabolism in young animals depends upon a more active decomposition in the cells than in older animals is still undecided.

As the total calories exchanged per kilo of body weight in young animals is greater than in older ones, this difference must be seen in measuring as well the exchange of gas as the elimination of nitrogen. This is true, and we give here CAMERER'S[1] figures on the elimination of urea in children.

TABLE XII.

Age.	Weight of Body in Kilos.	Urea in grms.	
		Per Day.	Per Kilo.
1½ years	10.80	12.10	1.35
3 "	13.30	11.10	0.90
5 "	16.20	12.37	0.76
7 "	18.80	14.05	0.75
9 "	25.10	17.27	0.69
12½ '	32.60	17.79	0.54
15 "	35.70	17.78	0.50

In adults weighing about 70 kilos about 30–35 grms. urea per day are eliminated, or 0.5 grm. per kilo. At about 15 years of age the destruction of proteids per kilo is about the same as in adults. The relatively greater metabolism of proteids in young individuals is explained partly by the fact that the metabolism of material in general is more active in young animals, and partly by the fact that young animals are as a rule poorer in fat than those full grown.

As the metabolism may be kept at its lowest point by absolute rest of body and inactivity of the intestinal tract, it is manifest that work and the taking up of food have an important bearing on the extent of metabolism.

Rest and *Work*. During work a greater quantity of potential energy is converted into living force, i.e., the metabolism is increased more or less on account of work.

As explained in a previous chapter (XI) work, according to the generally accepted view, has no material influence on the elimination of nitrogen. It is nevertheless true that several investigators in certain cases have observed an increased elimination of nitrogen; but these observations have been explained in other ways.

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[1] Zeitschr. f. Biologie, Bdd. 16 u. 20.

For instance, work may, when it is connected with violent movements of the body, easily cause dyspnœa, and this last, as **Fränkel**[1] has shown, since diminution of the oxygen supply increases the proteid metabolism, may cause an increase in the elimination of nitrogen. In other series of experiments the quantity of carbohydrates and fats in the food was not sufficient; the supply of fat in the body was decreased thereby, and the destruction of proteids was correspondingly increased. Work may also increase the appetite, and an increase in the elimination of nitrogen may be caused by the greater quantity of proteids taken. According to the generally accepted views muscular activity has hardly any influence on the metabolism of proteids.

On the contrary, work has a very considerable influence on the elimination of carbon dioxide and the consumption of oxygen. This action, which was first observed by **Lavoisier**, has recently been confirmed by many investigators. **Pettenkofer** and **Voit**[2] have made investigations on a full-grown man as to the metabolism of the nitrogenous as well as of the non-nitrogenous bodies during rest and work, partly while fasting and partly on a mixed diet. The experiments were made on a full-grown man weighing 70 kilos. The results are contained in the following table:

TABLE XIII.

		Consumption of				
		Proteids.	Fat.	Carbohydrates.	CO_2 eliminated.	O consumed.
Fasting...	Rest	79	209	...	716	761
	Work	75	380	...	1187	1071
Mixed diet	Rest	137	72	352	912	831
	Work	137	173	352	1209	980

In these cases work did not seem to have any influence on the destruction of proteids, while the gas exchange was considerably increased.

Zuntz and his pupils **Lehmann**[3] and **Katzenstein**[4] have made very important investigations on the extent of the exchange of gas as a measure of metabolism during work and caused by work, using **Zuntz-Geppert**'s method (see page 604). These investigations not only show the important influence of muscular work on the decomposition of material, but they also show in a very instructive way the relationship between the extent of metabolism of material and useful work of various kinds. We can only refer to these important investigations, which are of special physiological interest.

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[1] Virchow's Arch., Bdd. 67 u. 71.

[2] Zeitschr. f. Biologie, Bd. 2.

[3] Maly's Jahresber., Bd. 19, S. 412.

[4] Pflüger's Arch., Bd. 49.

The action of muscular work on the gas exchange does not alone appear with hard work. From the researches of **Speck**,[1] who has also made very meritorious studies on the exchange of gas in man under various conditions, we learn that even very small, apparently quite unessential movements may increase the production of carbon dioxide to such an extent that by not observing these, as in numerous older experiments, very considerable errors may creep in.

The quantity of carbon dioxide eliminated during a working period is uniformly greater than the quantity of oxygen taken up at the same time, and hence a raising of the respiratory quotient was formerly usually considered as caused by work. This rise does not seem to be based upon the kind of chemical processes going on during work, as we have a series of experiments made by **Zuntz**, **Lehmann**, and **Katzenstein** in which the respiratory quotient remained almost wholly unchanged in spite of work. According to **Loewy**[2] the combustion processes in the animal body go on in the same way in work as in rest, and a raising of the respiratory quotient (irrespective of the transient change in the respiratory mechanism) takes place only with insufficient supply of oxygen to the muscles, as in continuous fatiguing work or short excessive muscular activity, also with local lack of oxygen caused by excessive work of certain groups of muscles. This varying condition of the respiratory quotient has been explained by **Katzenstein**[3] by the statement that during work two kinds of chemical processes act side by side. The one depends upon the work which is connected with the production of carbon dioxide also in the absence of free oxygen, while the other brings about the regeneration which takes place by the taking up of oxygen. When these two chief kinds of chemical processes make the same progress the respiratory quotient remains unchanged during work; if by hard work the decomposition is increased as compared with the regeneration, then a raising of the respiratory quotient takes place.

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[1] Speck, Physiologie des menschlichen Athmens. Leipzig, 1892.

[2] Pflüger's Arch., Bd. 49.

[3] *Ibid.*, Bd. 49.

In *sleep* metabolism decreases as compared with that during waking, and the most essential reason for this is the muscular inactivity during sleep. The investigations of RUBNER[1] on a dog, and of LOEWY[2] on human beings, teach us that if the muscular work is eliminated the metabolism during waking is not greater than in sleep.

The action of *light* also stands in close connection to the question of the action of muscular work. It seems positively proven that metabolism is increased under the influence of light. Most investigators, such as SPECK,[3] LOEB,[4] and EWALD,[5] consider that this increase is due to the movements caused by the light or an increased muscle tonus. FUBINI and BENEDICENTI[6] assume that the increase in metabolism due to light is independent of the movements. They base this assumption on experiments made on hibernating animals.

Mental activity does not seem to have any influence on metabolism.

Action of the *External Temperature.* In cold-blooded animals the production of carbon dioxide increases and decreases with the rise and fall of the surrounding temperature. In warm-blooded animals this condition is the reverse. By the investigations of LUDWIG and SANDERS-EZN, PFLÜGER and his pupils, and Duke CHARLES THEODORE of Bavaria and others,[7] it has been demonstrated that in warm-blooded animals the change in the external temperature has different results according as the animal's own heat remains the same or changes. If the temperature of the animal sinks, the elimination of carbon dioxide decreases; if the temperature rises, the elimination of CO_2 increases. If, on the contrary, the temperature of the body remains unchanged, then the elimination of carbon dioxide increases with a lower and decreases with a higher external temperature. This fact may be explained, according to **Pflüger** and **Zuntz**, by the statement that the low temperature, by exciting a reflex action in the sensitive nerves of the skin, causes an increased metabolism in the muscles with an increased production of heat, affecting the temperature of the body, while with a higher external temperature the reverse takes place. The experiments made on animals are somewhat uncertain for several reasons, but the determinations of the oxygen absorption, as well as the elimination of CO_2, made by **Speck**[1] and **Loewy**[2] on human beings, have shown that cold does not produce any essential increase in the metabolism of man. The irritation caused by cold may reflexly cause a forced respiration with an action on the gas exchange, and weak reflex muscular movements, such as shivering, trembling, etc., may cause an insignificant increase in the elimination of carbon dioxide; in complete muscular inactivity cold seems to cause no increased absorption of oxygen or increased metabolism. According to **Loewy** the most essential thing in the regulation of heat under the influence of cold is, not an increased production of heat, but rather a diminished loss of heat by contraction of the skin and its vessels.

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[1] Ludwig-Festschrift, 1887.

[2] Berlin. klin. Wochenschr., 1891, S. 434.

[3] L. c.

[4] Pflüger's Arch., Bd. 42.

[5] Journal of Physiol., Vol. 13.

[6] See Maly's Jahresber., Bd. 22, S. 395.

[7] The pertinent literature may be found cited by Voit in Hermann's Handbuch, Bd. 6, and also by Speck, l. c.

Metabolism is increased by the *partaking of food*, and **Zuntz**[3] has calculated that in man the consumption of oxygen is raised on an average 15% for about 6 hours after taking a moderately hearty meal. This increase in the metabolism is caused, according to the generally accepted view of **Speck**, probably only by the increased work of the digestive apparatus on the partaking of food. **Fick**[4] claims that the increased metabolism is due to the oxidation of the circulating, combustible material (proteid). This view, as shown by **Magnus-Levy**,[5] is not correct; but still **Levy** inclines to the view that besides the digestion work the proteids may possibly also have a specific exciting action on metabolism.

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[1] L. c.

[2] Pflüger's Arch., Bd. 46.

[3] Zuntz and Levy, Beitrag zur Kenntniss der Verdaulichkeit, etc., des Brodes. Pflüger's Arch., Bd. 49.

[4] Sitzungsber. d. Würzb. phys.-med. Gesellsch., 1890.

[5] Pflüger's Arch., Bd. 55, contains the pertinent literature.

VI. The Need of Food by Man under Various Conditions.

Various attempts have been made to determine the daily quantity of organic food needed by man. Certain investigators have calculated, from the total consumption of food by a large number of similarly fed individuals, soldiers, sailors, laborers, etc., the average quantity of food required per head. Others have calculated the daily demand of food from the quantity of carbon and nitrogen in the excreta. Others again have calculated the quantity of nutritive material in a diet by which an equilibrium was maintained in the individual for one or several days between the consumption and elimination of carbon and nitrogen. Lastly, others still have quantitatively determined during a period of several days the organic nutritive substances consumed daily by persons of various occupations who chose their own food, by which they were well nourished and rendered fully capable of labor.

Among these methods a few are not quite free from reproach, and others have not as yet been tried on a sufficiently large scale. Nevertheless the experiments collected thus far serve, partly because of their number and partly because of the methods, to correct and control one another, and also serve as a good starting-point in determining the diet of various classes and similar questions.

If the quantity of nutritive substance taken daily be converted into calories produced during physiological combustion, we then obtain some idea of the sum of the chemical potential energy which under varying conditions is introduced into the body. It must not be forgotten that the food is never completely absorbed, and that undigested or unabsorbed residues are always expelled from the body with the fæces. The gross results of calories calculated from the food taken must therefore, according to RUBNER,[1] be diminished at least 8%.

The following summary contains certain examples of the quantity of food which is consumed by individuals of various classes under different conditions. In the last column we also find the quantity of living force which corresponds to the quantity of food in question, calculated as calories, with the above-stated correction.

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[1] Zeitschr. f. Biologie, Bd. 21, S. 379.

The calories are therefore net results, while the figures for the nutritive bodies are gross results.

TABLE XIV.

	Proteids.	Fat.	Carbohydrates.	Calories.	Authority.
Soldier during peace....	119	40	529	2784	PLAYFAIR.[1]
" light service.....	117	35	447	2424	HILDESHEIM.
" in field....	146	46	504	2852	"
Laborer.................	130	40	550	2903	MOLESCHOTT.
" at rest	137	72	352	2458	PETTENKOFER & VOIT.
Cabinet-maker (40 years).	131	68	494	2835	FORSTER.[2]
Young physician........	127	89	362	2602	"
" "	134	102	292	2476	"
Laborer	133	95	422	2902	"
English smith...........	176	71	666	3780	PLAYFAIR.
" pugilist.........	288	88	93	2189	"
Bavarian wood-chopper..	135	208	876	5589	LIEBIG.
Laborer in Silesia......	80	16	552	2518	MEINERT.[3]
Seamstress in London...	54	29	292	1688	PLAYFAIR.
Swedish laborer........	134	79	485	3019	HULTGREN & LANDERGREN.[4]
Japanese student........	83	14	622	2779	EIJKMAN.[5]
" shopman......	55	6	394	1744	TAWARA.[5]

It is evident that persons of essentially different weight of body who live under unequal external conditions must need essentially different food. It is also to be expected (and this is confirmed by the table) that not only the absolute quantity of food consumed by various persons, but also the relative proportion of the various organic nutritive substances, shows considerable variation. Results for the daily need of human beings in general cannot be given. For certain classes of human beings, such as soldiers, laborers, etc., results may be given which are valuable for the calculation of the daily rations.

Based on extensive investigations and a very wide experience, VOIT has proposed the following average quantities for the daily diet of adults:

	Proteids.	Fat.	Carbohydrates.	Calories.
For men..........	118 grms.	56 grms.	500 grms.	2810

But it should be remarked that these statements relate to a man weighing 70 to 75 kilos and who was engaged daily for ten hours in not too fatiguing labor.

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[1] In regard to the older researches cited in this table we refer the reader to Voit in Hermann's Handbuch, Bd. 6, S. 519.

[2] *Ibid.* and Zeitschr. f. Biologie, Bd. 9.

[3] Armee- und Volksernährung. Berlin, 1880.

[4] Investigations on the food of Swedish laborers with free selected diet. Stockholm, 1891.

[5] Cited from Kelner and Mori in Zeitschr. f. Biologie, Bd. 25.

The quantity of food required by a woman engaged in moderate work is about ⅘ that of a laboring man, and we may consider the following as a daily diet with moderate work:

	Proteids.	Fat.	Carbohydrates.	Calories.
For women.......	94 grms.	45 grms.	400 grms.	2240

The proportion of fat to carbohydrates is here as 1 : 8–9. Such a proportion occurs often in the food of the poorer classes, while the ratio in the food of wealthier persons is 1 : 3–4. The maximum quantity of carbohydrates in the food must, according to VOIT, not be above 500 grms.; and as the carbohydrates besides constitute the chief part of the often very bulky vegetable foods, it has been suggested and is desirable on this and other grounds to increase the quantity of fat at the expense of the carbohydrates in such rations. But because of the high price of fat such a modification cannot always be made.

In examining the above numbers for the daily rations it must not be forgotten that the figures for the various nutritive bodies are gross results. They consequently represent the quantity of the nutritive bodies which must be taken in, and not those which are really absorbed. The figures for the calories are, on the contrary, net results.

The various foods are, as is well known, not equally digested and absorbed, and in general the vegetable foods are less completely used up than animal foods. This is especially true of the proteids. When, therefore, VOIT, as above stated, calculates the daily quantity of proteids needed by a laborer as 118 grms., he starts with the supposition that the diet is a mixed animal and vegetable one, and also that of the above 118 grms. about 105 grms. are absorbed. The results obtained by PFLÜGER and his pupils BLEIBTREU[1] and BOHLAND[2] for the extent of the metabolism of proteids in man with an optional and sufficient diet correspond well with the above figures, when the unequal weight of body of the various persons experimented upon is sufficiently considered.

As a rule, the more exclusively a vegetable food is employed, the smaller is the quantity of proteids in the same. The strictly vegetable diet of certain people, as of the Japanese and that of the so-called vegetarians, is therefore a proof that, if the quantity of food be sufficient, a person may exist on considerably smaller quantities of proteids than Voit suggests. It follows from the investigations of Hirschfeld, Kumagawa, and Klemperer (see page 638) that a nearly complete or indeed a complete nitrogenous equilibrium may be attained by the sufficient administration of non-nitrogenous nutritive bodies with relatively very small quantities of proteids.

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[1] Pflüger's Arch., Bd. 36.

[2] *Ibid.*, Bd. 38.

If we bear in mind that the food of people of different countries varies greatly, and that the individual also takes essentially different nourishment according to the external conditions of living and the influence of climate, it is not remarkable that a person accustomed to a mixed diet cannot exist for a long time on a strictly vegetable diet deficient in proteids, even though not especially difficult to digest. No one doubts the ability of man to adapt himself to a heterogeneously composed diet when this is not too difficult of digestion and is sufficient; but this ability does not seem sufficient reason for essentially altering the figures suggested by Voit. Although man may be satisfied under certain circumstances with a lower quantity of proteid than that calculated by Voit, still it does not follow that such a diet is also the most serviceable. Voit's figures are only given for certain cases or certain categories of human beings. It is apparent that other figures must be taken for other cases, and it is evident that the daily ration given by Voit as necessary for a laborer must be altered slightly for other countries because of the existing conditions in middle Europe, where Voit made his investigations. For example, Hultgren and Landergren have shown in very careful investigations that the laborer in Sweden with moderate work and an average body weight of 70.3 kilos, with optional diet, partakes 134 grms. proteid, 79 grms. fat, and 522 grms. carbohydrates. The quantity of proteid partaken of is here greater than is necessary according to Voit.

If we compare the figures of Table XIV with the average figures proposed by Voit for the daily diet of a laborer, it would seem at the first glance as if the consumed food in certain cases was considerably in excess of the need, while in other cases, as for instance for the seamstress in London, it was entirely insufficient. A positive conclusion cannot, therefore, be drawn if we do not know the weight of the body, as well as the labor performed by the person, and also the conditions of living. It is certainly true that the amount of nutriment required by the body is not directly proportional to the bodily weight, for a small body consumes relatively more substance than a larger one, and varying quantities of fat may also cause a difference; but a large body, which must maintain a greater quantity, consumes an absolutely greater quantity of substance than a small one, and in estimating the nutritive need one must also always consider the weight of the body. According to Voit, the diet for a laborer with 70 kilos bodily weight requires 40 calories for each kilo.

As several times stated above, the demands of the body for nourishment vary with its varying conditions. Among these conditions two are especially important, namely, labor and rest.

In a previous chapter, in which muscular labor was spoken of, it was seen that the generally accepted view is that non-nitrogenous food is the most essential, if not the exclusive, source of muscular force. As a natural sequence it is to be expected that in activity the non-nitrogenous foods before all must be increased in the daily rations.

Still this does not seem to hold true in daily experience. It is a well-known fact that hard-working individuals—men and animals—require a greater quantity of proteids in the food than less active ones. This contradiction is, however, only apparent, and it depends, as Voit has shown, upon the fact that individuals used to violent work are more muscular. For this reason a person performing severe muscular labor requires food containing a larger proportion of proteids than an individual whose occupation demands less violent exertion. Another question is, how should the relative and absolute quantity of food be changed if increased exertion be demanded of one and the same individual?

An answer based upon experience may be found in statistics concerning the maintenance of soldiers in peace and in war. Many such statements are obtainable. In a critical examination of the same it is found that in war rations the quantity of non-nitrogenous bodies as compared to the proteids is only increased in exceptional cases, while usually the reverse is the case. Even in these cases the actual proportion does not correspond with the theoretical demand, upon which, however, too great stress must not be placed, since in the case of soldiers in the field many other circumstances are to be considered, such as the volume and weight of the food, etc., etc., which cannot here be more closely discussed. The following table shows the average results of soldiers' rations in war and peace from the data given for various countries.[1] These average results also include the figures for Sweden.

TABLE XV.

	A. Peace Ration.			B. War Ration.		
	Proteids.	Fat.	Carb.	Proteids.	Fat.	Carb.
Minimum	108	22	504	126	38	484
Maximum	165	97	731	197	95	688
Mean	130	40	551	146	59	557
Sweden (proposed)	179	102	591	202	137	565

If we do not consider the very abundant rations proposed for the soldier in Sweden, and if we only adhere to the above mean figures, we obtain the following results for the daily rations:

	Proteids.	Fat.	Carb.	Calories.
In peace	130	40	551	2900
In war	146	59	557	3250

If we calculate the fat in its equivalent quantity of starch, then the relation of the proteids to the non-nitrogenous foods is:

In peace	1:4.97
In war	1:4.79

The proportion is nearly the same in both cases; the slight difference which occurs shows a trifling relative increase in the proteids in the war ration. On the contrary, as is especially apparent from the total of the calories, the total quantity of nutritive bodies is greater in the war than in the peace ration.

As more work requires an increase in the absolute quantity of food, so the quantity of food must be diminished when little work is performed. The question as to how far this can be done is of importance in regard to the diet in prisons and poorhouses. We give below the following as example of such diets:

TABLE XVI.

	Proteids.	Fat.	Carb.	Calories.	
Prisoner (not working)	87	22	305	1667	SCHUSTER.[2]
" " "	85	30	300	1709	VOIT.
Man in poorhouse	92	45	332	1985	FORSTER.[3]
Woman in "	80	49	266	1725	"

The figures given by VOIT are, according to him, the lowest figures for a non-working prisoner. He considers the following as the lowest diet for old non-working people:

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[1] Germany, Austria, Switzerland, France, Italy, Russia, and the United States.

[2] See Voit, Untersuchung der Kost. München, 1877. S. 142.

[3] *Ibid.*, S. 186.

	Proteids.	Fat.	Carb.	Calories.
Men	90	40	350	2200
Women	80	35	300	1733

In calculating the daily diet it is in most cases sufficient to ascertain how much of the various nutritive substances must be daily administered to the body to keep it in the proper condition to perform the work required of it. In other cases it may be a question of improving the nutritive condition of the body by properly selected food; but we also have cases in which we desire to diminish the mass or weight of the body by an insufficient nutrition. This is especially the case in obesity, and all the dietaries proposed for this purpose are chiefly starvation cures.

The oldest and most generally known diet cure for corpulency is that of Harvey,[1] which is ordinarily called the Banting method. The principle of this cure consists in increasing, as far as possible, the consumption of the accumulated fat of the body by as limited a supply of fat and carbohydrates as possible and a simultaneous increased supply of proteids. A second cure, called Ebstein's[2] cure, is based on the assumption (not correct) that the fat of the food is not accumulated in a body rich in fat, but is completely burnt. In this cure large quantities of fat are therefore allowed in the food, while the quantity of carbohydrates is diminished very materially. The third cure, called Oertel's[3] cure, is based on the correct view that a certain quantity of carbohydrates has no greater influence in the accumulation of fat than the isodynamic quantities of fat. In this cure, therefore, carbohydrates as well as fat are allowed, provided the total quantity of the same is not so great as to hinder the decrease in the fatty condition. A greatly diminished supply of water is also one of the features of Oertel's cure, especially in certain cases. The average quantity of the various nutritive substances supplied to the body in these three cures is as follows, and we give also for comparison in the same table Voit's diet necessary for a laborer:

	Proteids.	Fat.	Carb.	Calories.
Harvey-Banting's cure	171	8	75	1066
Ebstein's cure	102	85	47	1391
Oertel's "	156	22	72	1124
" " (max.).	170	44	114	1557
Laborer, according to Voit	118	56	500	2810

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[1] Banting, Letter on Corpulence. London, 1864.

[2] Ebstein, Die Fettliebigkeit und ihre Behandlung. 1882.

[3] Oertel, Handbuch der allg. Therapie der Kreislaufstörungen. 1884.

If the fat in all cases is recalculated in starch, then the proportion of the proteids to the carbohydrates is:

Harvey-Banting's cure	100 : 54
Ebstein's cure	100 : 246
Oertel's "	100 : 80
" " (max.)	100 : 129
Laborer	100 : 540

In all these cures for corpulence the quantity of non-nitrogenous bodies is diminished as compared with the proteids; but chiefly the total quantity of food, as is shown by the number of calories, is considerably diminished.

Harvey-Banting's cure differs from the others in a relatively very much greater quantity of proteids, while the total number of calories in it is the smallest. On this account this cure acts very quickly; but it is therefore also more dangerous and more difficult to accomplish. In this regard Ebstein's and Oertel's cures (especially Oertel's), having a greater variation in the selection of food, are better. As the adipose tissue has a proteid-sparing action, we have to consider in using these cures, especially Banting's, that the destruction of proteids in the body is not increased with the decrease in the adipose tissue, and one must therefore carefully watch the elimination of nitrogen by the urine. All diet cures for obesity are moreover, as above stated, starvation cures; and if the daily quantity of food required by an adult man, represented as calories, is in round numbers 2500 calories (according to the average figures found by Forster in the case of a physician), then one immediately sees what a considerable part of its own mass the body must daily give up in the above cures. This reminds us of the great care necessary in employing these cures; but each special case should be conducted with regard to the individuality, the weight of the body, the elimination of nitrogen in the urine, etc., etc., and always under strong control and only by physicians, never by a layman. A closer discussion of the many conditions which must be considered in these cases does not enter into the plan and scope of this work.

TABLE I.—FOODS.[1]

1. Animal Foods.	1000 Parts contain						Relationship of		
	1 Proteids and Extractives.	2 Fat.	3 Carbohydrates.	4 Ash.	5 Water.	6 Waste.	1	: 2	: 3
a. FLESH WITHOUT BONES.									
Fat beef[2]	183	166		11	640		100	90	0
Beef (average fat[3])	196	98		18	688		100	50	0
Beef[2]	190	120		18	672		100	63	0
Corned beef (average fat)	218	115		117	550		100	53	0
Veal	190	80		13	717		100	42	0
Horse, salted and smoked	318	65		125	492		100	20	0
Smoked ham	255	365		100	280		100	143	0
Pork, salted and smoked[4]	100	660		40	130		100	660	0
Flesh from hare	233	11		12	744		100	5	0
" " chicken	195	93		11	701		100	48	0
" " partridge	253	14		14	719		100	6	0
" " wild duck	246	31		12	711		100	13	0
b. FLESH WITH BONES.									
Fat beef[2]	156	141		9	544	150	100	90	0
Beef, average fat[3]	167	83		15	585	150	100	49	0
Beef, slightly corned	175	93		85	480	167	100	53	0
Beef, thoroughly corned	190	100		100	430	180	100	53	0
Mutton, very fat	135	332		8	437	88	100	246	0
" average fat	160	160		10	520	150	100	100	0
Pork, fresh, fat	100	460		5	365	70	100	460	0
Pork, corned, fat	120	540		60	200	80	100	450	0
Smoked ham	200	300		70	340	90	100	150	0
c. FISHES.									
River eel, fresh, entire	89	220		6	352	333	100	246	0
Salmon, " "	121	67		10	469	333	100	56	0
Anchovy, " "	128	39		11	489	333	100	31	0
Flounder, " "	145	14		11	580	250	100	9	0
River perch, " "	100	2		8	440	450	100	2	0
Torsk, " "	86	1		8	455	450	100	1	0

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[1] The results in the following tables are chiefly compiled from the summary of ALMÉN and of KÖNIG. As "waste" we here designate that part of the foods which is lost in the preparation of the food or that which is not used by the body; for instance, the bones, skin, egg-shell, and the cellulose in the vegetable foods.

[2] Meat such as is ordinarily sold in the markets in Sweden.

[3] Beef such as is delivered by large purveyors to public institutions in Sweden.

[4] Pork, chiefly from the breast and belly, such as occurs in the rations of Swedish soldiers.

TABLE I.—FOODS.—(*Continued.*)

Animal Foods.	1000 Parts contain						Relationship of		
	1 Proteids and Extractives.	2 Fat.	3 Carbohydrates.	4 Ash.	5 Water.	6 Waste.	1	: 2	: 3
Pike, fresh, entire	82	1		6	461	450	100	1	0
Herring, salted, entire	140	140		100	280	340	100	100	0
Anchovy, " "	116	43		107	334	400	100	37	0
Salmon (side), salted	200	108		132	460	100	100	54	0
Kabeljau (salted haddock)	246	4		178	472	100	100	1	0
Codfish (dried ling)	532	5		106	257	100	100	1	0
" (dried torsk)	665	10		59	116	150	100	1	0
Fish-meal from variety of GADUS	736	7		87	170		100	1	0
d. INNER ORGANS (FRESH).									
Brain	116	103		11	770		100	89	0
Beef-liver	196	56	11	17	720		100	28	6
Beef-heart	184	92		10	714		100	50	0
Heart and lungs of mutton	163	106		10	721		100	65	0
Veal-kidney	221	38		13	728		100	17	0
Ox-tongue (fresh)	150	170		10	670		100	113	0
Blood from various animals (average results)	182	2		9	807		100	1	0
e. OTHER ANIMAL FOODS.									
Kind of pork-sausage (Mettwurst)	190	150		50	610		100	79	0
Same for frying	220	160		55	565		100	73	0
Butter	7	850	7	15	119		100	12100	100
Lard	3	990			7		100	33000	0
Meat extract	304			175	217				
Cow's milk (full)	35	35	50	7	873		100	100	143
" " (skimmed)	35	7	50	7	901		100	20	143
Buttermilk	41	9	38	7	905		100	22	93
Cream	37	257	35	6	665		100	695	95
Cheese (fat)	230	270	40	60	400		100	117	17
" (poor)	334	66	50	50	500		100	19	15
Whey cheese (poor)	89	70	456	56	329		100	79	512
Hen's egg, entire	106	93	4	8	654	135	100	88	4
" " without shell	122	107	5	10	756		100	88	4
Yolk of egg	160	307		13	520		100	192	0
White " "	103	7	7	8	875		100	7	7

TABLE I.—FOODS.—(*Continued.*)

2. Vegetable Foods.	1000 Parts contain						Relationship of		
	1 Proteids and Extractives.	2 Fat.	3 Carbohydrates.	4 Ash.	5 Water.	6 Waste.	1	:2	:3
Wheat (grains)	123	17	676	18	140	26	100	14	549
Wheat-flour (fine)	110	10	740	8	120	2	100	11	654
" (very fine)	92	11	768	3	120	6	100	12	835
Wheat-bran	150	39	439	50	130	192	100	26	292
Wheat-bread (fresh)	88	10	550	17	330	5	100	11	625
Macaroni	90	3	768	8	131		100	3	853
Rye (grains)	115	17	688	18	140	22	100	15	600
Rye-flour	115	15	720	20	110	20	100	13	626
Rye-bread (dry)	114	20	725	15	110	16	100	18	634
" " (fresh, coarse)	77	10	480	16	400	17	100	14	623
" " (fresh, fine)	80	14	514	11	370	11	100	18	634
Barley (grains)	111	21	654	26	140	48	100	19	589
Scotch barley	110	10	720	7	146	7	100	9	654
Oat (grains)	117	60	563	30	130	100	100	51	481
Oat (peeled)	140	60	660	20	100	20	100	43	471
Corn	101	58	656	17	140	28	100	57	662
Rice (peeled for boiling)	70	7	770	2	146	5	100	10	1100
French beans	232	21	537	36	137	37	100	9	231
Peas (yellow or green)	220	15	580	25	150	60	100	7	240
Flour from peas	270	15	520	25	125	45	100	6	192
Potatoes	20	2	200	10	760	8	100	10	1030
Turnips	14	2	74	7	893	10	100	14	529
Carrot (yellow)	10	2	90	10	873	15	100	20	900
Cauliflower	25	4	50	8	904	9	100	16	200
Cabbage	19	2	49	12	900	18	100	11	258
Beans	27	1	66	6	888	12	100	4	244
Spinach	31	5	33	19	908	8	100	16	106
Lettuce	14	3	22	10	944	7	100	21	157
Cucumbers	10	1	23	4	956	6	100	10	230
Radishes	12	1	38	7	934	8	100	8	317
Edible mushrooms (average)	32	4	60	9	877	18	100	12	188
Same dried in the air (average)	219	25	412	61	160	123	100	12	188
Apples and pears	4		130	3	832	31	100		3250
Various berries (average)	5		90	6	849	50	100		1800
Almonds	242	537	72	29	54	66	100	222	30
Cocoa	140	480	180	50	55	95	100	343	129

TABLE II.—MALT LIQUORS.

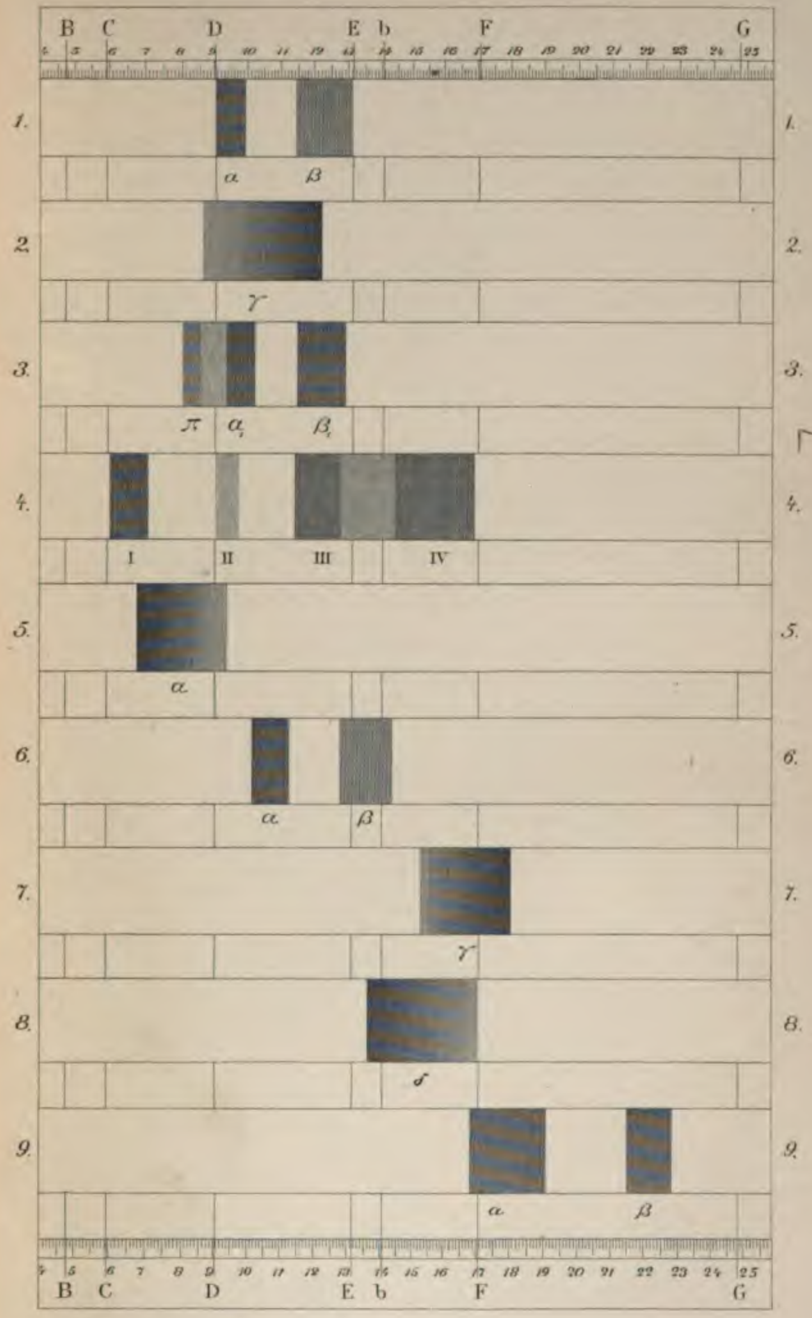
1000 Parts by Weight contain	Water.	Carbon Dioxide.	Alcohol.	Extract.	Proteids.	Sugar.	Dextrin.	Acids.	Glycerine.	Ash.
Porter	871	2	54	76	7	13		3	—	4
Beer (Swedish)	887		28	—	15	65		—	—	5
" (Swedish export)	885		32	—	7	73		—	—	3
Draught-beer	911	2	35	55	8	10	31	2	2	2
Lager-beer	903	2	40	58	4	7	47	1.5	2	2
Bock-beer	881	2	47	72	6	13	—	1.7	—	3
Weiss-beer	916	3	25	59	5	—	—	4	—	2
Swedish "Svagdricka"	945	—	22		7	23		—	—	3

TABLE III.—WINE AND OTHER ALCOHOLIC LIQUORS.

1000 Parts by Weight contain	Water.	Alcohol, Vol. per cent.	Extract.	Sugar.	Acid and Potassium Bitartrate.	Glycerine.	Ash.	Carbon Dioxide, Vol. per cent.
Bordeaux wine	883	94	23	6	5.9		2.0	
White wine (Rheingau)	863	115	23	4	5.0		2.0	
Champagne	776	90	134	115	6.0	1.0	1.0	60–70
Rhine wine (sparkling)	801	94	105	87	6.0	1.0	2.0	
Tokay	808	120	72	51	7.0	9.0	3.0	
Sherry	795	170	35	15	5.0	6.0	5.0	
Port-wine	774	164	62	40	4.0	2.0	3.0	
Madeira	791	156	53	33	5.0	3.0	3.0	
Marsala	790	164	46	35	5.0	4.0	4.0	
Swedish punch	479	263		332				
Brandy		460						
French cognac		550						
Liqueurs		442–590		260–475				

SPECTRUM PLATE.

1. Absorption spectrum of a solution of *oxyhæmoglobin.*
2. Absorption spectrum of a solution of *hæmoglobin,* obtained by the action of an ammoniacal ferro-tartrate solution on an oxyhæmoglobin solution.
3. Absorption spectrum of a faintly-alkaline solution of *methæmoglobin.*
4. Absorption spectrum of a solution of *hæmatin* in ether containing oxalic acid.
5. Absorption spectrum of an alkaline solution of *hæmatin.*
6. Absorption spectrum of an alkaline solution of *hæmochromogen,* obtained by the action of an ammoniacal ferro-tartrate solution on an alkaline-hæmatin solution.
7. Absorption spectrum of an acid solution of *urobilin.*
8. Absorption spectrum of an alkaline solution of *urobilin* after the addition of a zinc-chloride solution.
9. Absorption spectrum of a solution of *lutein* (ethereal extract of the egg-yolk).


B C D E b F G
4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
1.
α β
2.
γ
3.
π α₁ β₁
4.
I II III IV
5.
α
6.
α β
7.
γ
8.
δ
9.
α β

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